zerumbone (*Zingiber zerumbet* Smith, Zingiberaceae), yakuchinone A and B (*Alpinia oxyphylla* Miquel, Zingiberaceae), eupatiline (Artemia asiatica Nakai), etc. The chemical structures of some of these compounds and their sources are illustrated in Figure. A trans-disciplinary endeavor called 'reverse pharmacology' has recently emerged, and this new academic discipline can reduce major bottlenecks (cost, time and toxicity) frequently encountered in conventional drug development. Reverse pharmacology offers a major paradigm shift in drug discovery. Instead of serendipitous findings persued randomly, an organized path from clinical experiences, experimental observations and data base has been extablished. CAM markets are rapidly growing, and there are ample opportunities of research for the development of botanicals as pharmaceuticals as well as nutraceuticals. This work was supported by the Global Core Research Program (GCRC) from the National Research Foundation (NRF), Ministry of Education, Science and Technology, Republic of Korea.

Keywords: inflammation, chemoprevention, phytochemicals

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Natural Compounds as Inhibitors of the 10 Hallmarks of Cancer

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Cancer is one of the most deadly diseases in the world. Although advances in the field of chemo-preventive and therapeutic medicine have been made regularly over the last ten years, the search for novel anticancer treatments continues. In this field, the natural environment, with its rich variety of organisms, is a largely untapped source of novel compounds with potent antitumor activity. We focus here on selected compounds that act on the eight major hallmarks of cancer including self-sufficiency in growth signals, insensitivity to anti-growth signals, evasion of apoptosis, limitless replication, sustained angiogenesis and tissue invasion, metastasis, altered cellular metabolism and the evasion of immune destruction. Moreover, we identify compounds that interfere with the two enabling characteristics coined by Hanahan and Weinberg recently, namely inflammation and genome instability [1].

The definition of a natural compound is very complex. Traditionally, a natural product is a chemical compound produced by living organisms and possessing biological or pharmacological activity. Moreover a natural product can also be synthesized and thus be chemically identical to its natural counterpart. In order to elucidate the contribution of natural products in chemotherapeutic drug discovery and development, Newman and Cragg generated a drug classification after evaluation of all approved anticancer drugs between 1940 and 2010. In their analysis, 206 approved anticancer agents were classified into clearly defined groups [2].

Use of natural products has limitations as living organisms sometimes synthetize only trace quantities of otherwise interesting bioactive compounds. Another aspect is the limited bioactivity of natural products. Here, the polarity of the molecule often complicates its cellular uptake, thus leading to a reduced activity. Therefore optimization of natural products by removal, introduction or modification of functional groups of active natural product scaffolds in order to improve their bioactivity on one hand, together with natural product-inspired combinatorial synthesis providing large libraries of compounds in a short time on the other hand are the promising strategies to obtain powerful drug leads [3].

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Keywords: cancer, natural compounds, chemoprevention, therapy

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Mangifera indica Stem Bark Extract (Vimang) and Its Main Polyphenol Mangiferin: From the Ethnomedicine as Natural Supplements to the Preclinical and Clinical Investigations for New Phytomedicines

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Cuba, an island characterized by a rich plant and marine biodiversity, with very high index of endemic flora, offers to public health important therapeutic alternatives. Different projects of clinical investigations with natural products should concluded in the controlled clinical trials, and pharmacoepidemiological studies, in order to known the real effectively of medicinal plant as part of therapeutic police of the Ministry of Health.

Many examples demonstrated the utilization of natural product from tropical plants in Cuba. In particular, we present the result of investigations with an aqueous extract from stem bark of *Mangifera indica* L (VIMANG) that has been used in Cuba during several years in ethnomedical practices. Phytochemical characterization of the extract has led to the isolation of different phenolic constituents, with the glucosylxanthone mangiferin as the majority component. The extract has demonstrated antioxidant activity as the main pharmacological property. Others studies have shown that the extract also possesses others pharmacological activities, such as: anti-inflammatory, antiallergic, analgesic, neuroprotector and immunomodulator with very complex and multifactorial mechanisms involved in its action. Different clinical studies in dermatitis, asthma, diabetes, arthritis, have been developed, demonstrating the therapeutic effectiveness of Vimang as antioxidant supplement in pathologies where oxidative stress is related with their etiology. This is only an example that illustrates the impact of the Cuban ethnomedicine in the national politics of health, on the base of the rational employment and with a deep scientific support, of the medicinal plants as true therapeutic alternatives. (Nuñez-Selles A et al. *Pharmacol Res* 2007;55: 351-358).

Essential Role for Cardiomyocyte Glucocorticoid Receptors in the Prevention of Heart Disease

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Glucocorticoids are primary stress hormones essential for life that have been implicated in cardiovascular health and disease. Whether these cardiovascular effects reflect systemic actions of glucocorticoids and/or direct actions of these steroids on cardiomyocytes is poorly understood. Glucocorticoids bind and activate the glucocorticoid receptor (GR), a member of the nuclear receptor superfamily of ligand-dependent transcription factors. Upon glucocorticoid occupancy, GR regulates the expression of thousands of genes by direct binding to DNA and/or by interactions with other chromatin-bound transcription factors. To determine the *in vivo* role of glucocorticoid signaling in the heart, we developed mice with cardiomyocyte-specific deletion of GR. The cardioGRKO mice appear normal early in life but die prematurely from spontaneous cardiovascular disease. By three months of age, the cardioGRKO mice display a marked reduction in left ventricular systolic function, as evidenced by decreases in ejection fraction and fractional shortening. Heart weight and left ventricular mass are also elevated in the cardioGRKO mice, and cardiomyocyte size is increased. Global gene expression analysis of knockout hearts prior to pathology onset revealed aberrant regulation of over 100 genes associated with cardiovascular disease. Expression levels of genes important for cardiac contractility and for repressing cardiac hypertrophy were reduced in cardioGRKO mouse hearts deficient in GR signaling. These findings demonstrate that a deficiency in cardiomyocyte glucocorticoid signaling leads to altered gene expression profiles, spontaneous cardiac hypertrophy, heart failure and death, revealing for the first time an obligate role for GR in maintaining normal heart function. Moreover, they suggest that activation of cardiomyocyte GR may provide a new therapeutic approach for treating heart disease.

New Molecular Mechanisms Involved in the Immune Actions of Glucocorticoids

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Considering the importance of Th1/Th2 balance and Th17 regulation in the final outcome of immune and inflammatory responses, we analyzed how selective Glucocorticoid receptor (GR) modulation differentially regulates the activity of T master drivers of T cell differentiation, Transcription factors T-bet and GATA-3 are pivotal for the acquisition of Th1 or Th2 phenotype, respectively. Glucocorticoids (GC) regulate both Th1 and Th2 cytokines and favor a shift towards Th2 differentiation. We described for the first time GC regulation of these master TF. GC inhibits T-bet transcriptional activity by a transrepression mechanism involving glucocorticoid receptor (GR) physical interaction with T-bet. This interaction also blocks GR-dependent transcription. The mechanism underlying T-bet inhibition further involves reduction of T-bet binding to DNA. The first zinc finger region of GR is required for T-bet inhibition. The inhibition of T-bet by GC plays an important role in mediating the inhibition of IFN-γ gene expression, hallmark of Th1-mediated immunity. GC also inhibits GATA-3 transcriptional activity. This mechanism does not involve physical interaction between the GR and GATA-3, nor reduction of GATA-3 binding to DNA, as described for T-bet. Instead, GC inhibit GATA-3 activity by inhibition of p38 MAPK induced GATA-3 phosphorylation. Finally, GATA-3 inhibition impacts on interleukin-5 gene, a central Th2 cytokine. The dissociating non-steroidal GR ligand Compound A (CpdA), inhibits T-bet activity via a transrepressive mechanism, but different from GCs, CpdA induces GATA-3 activity by p38 MAPK induction. The GR inhibits ROR gamma t transcriptional activity through GR-ROR gamma t protein interaction and histone deacetylation at the main non coding sequence (CNS) 2 that regulates IL-17 expression. GR activity is modulated by post-translational modifications including phosphorylation, acetylation, ubiquitination, and SUMOylation. The GR has three SUMOylation sites: lysine (K) 297 and K313 in the N-terminal domain (NTD) and K721 within the ligand binding domain (LBD). SUMOylation of the NTD sites are responsible for a negative effect on the GR activity. RSUME (RWD-containing SUMOylation Enhancer) interacts with GR and increases its SUMOylation and uncovers a positive role for the third SUMOylation site, K721, on GR-mediated transcription. The action of RSUME involves also the inhibition of the NF-kB signaling pathway, through the regulartion of IkB SUMOylation. These different molecular mechanisms provide a rationale for dissecting GC targeting on Th1/Th2/Th17 cells and its role in autoimmune processes.

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Keywords: glucocorticoid receptor, transcription factors, T-helper cells, SUMOylation, inflammation

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Tissue-specific Action of Glucocorticoids

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Physiological fluctuations of circulating glucocorticoids are known to be important in a variety of biological processes, including maintenance of systemic homeostasis of metabolism, water-electrolyte handling, immune modulation, and stress response. Moreover, glucocorticoids have been use as a therapeutic agent, with significant beneficial outcomes in terms of treating, for example, inflammatory disorders and autoimmune diseases. Particularly when given at high doses and for extended periods of time, glucocorticoids are associated with multiple and potentially debilitating adverse outcomes, which are often chronic in nature and difficult to treat. However, molecular details of those glucocorticoid actions and side effects remain largely unknown.

Glucocorticoid actions are believed to be mediated its cognate intracellular receptor glucocorticoid receptor (GR). GR, acting as a ligand-inducible transcription factor, elicits modulation of gene expression. Given this, multiple actions of glucocorticoids can be understood from their tissue-specific effects on gene expression, either quantitatively or qualitatively. In this line, we challenged to identify GR tarfet genes and clarify their function in glucocorticoid-target organs. In this talk, our results from heart and skeletal muscle will be presented.

The Origin and History of Compound A

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Plants belonging to the genus Salsola (Family: Chenopodiaceae) are often found in the arid and semi-arid regions of our planet. In Southern Africa no less than 69 different Salsola species are known to occur in Namibia and the Republic of South Africa. This genus is mentioned in Bushmen folklore as a traditional medicine and Bushmen women have used aqueous extracts of Salsola as an oral contraceptive. In 1961 de Lange described a rare syndrome of prolonged gestation that occurred sporadically among Karakul sheep on a number of farms in the Keetmanshoop district in the southern region of Namibia [1]. The syndrome was also called "Grootlamsiekte" that can literally be translated to "Big Lamb Disease". It is characterised by a gestation period of up to 213 days (normal gestation 149 days) and fetal post maturity. The affected lambs present with an overgrown hair coat that renders them economically worthless to the Swakara industry.

It was later discovered that ingestion of the Namibian shrub, *Salsola tuberculatiformis* Botsch., by pregnant Karakul sheep, gave rise to the prolonged gestation and fetal post maturity and an investigation into the active agents in the plant, responsible for this syndrome, was subsequently initiated.

Initial investigations into the compounds from *S. tuberculatiformis* Botsch. that could lead induce prolonged gestation and foetal postmaturity, were hampered by the fact that the syndrome could not be reproduced in smaller laboratory animals. It was, however, discovered that the shrub acted as a contraceptive in rats and we decided to concentrate on the contraceptive properties, rather than prolonged gestation, as a possible assay system for active substances in the plant. A bioassay, based on the daily inspection of vaginal smears from female rats according to the method of Zarrow et al. [2], was subsequently designed to identify possible active agents in the shrub. Using this assay we could extract the contraceptive principle(s) with methanol. Further fractionation of the active extract, using standard solvent partitioning and chromatographic procedures, however, lead to rapid autocatalytic decomposition and polymerisation of the active compound(s) under these conditions. All the contraceptive activity could, however, be removed from active plant extracts by treatment with trimethylammonium acetyl hydrazide chloride (Girard-T reagent) and the derivatives formed in this manner were isolated by solvent partitioning and decomposed under acidic conditions to yield three substances identified as 4-hydroxy-acetophenone (1-(4-Hydroxy-phenyl)-ethanone, Ia) and 4-hydroxy-3-methoxy-acetophenone (1-(4-Hydroxy-3-methoxy-phenyl)-ethanone, Ib) and 4-hydroxybenzaldehyde (Ic). Benzaldehyde (Ic) was not biologically active and although both ketones showed some biological activity in rats, neither Ia nor Ib, nor a mixture of the two compounds could, at the levels found in the extract, account for the contraceptive activity of the plant.

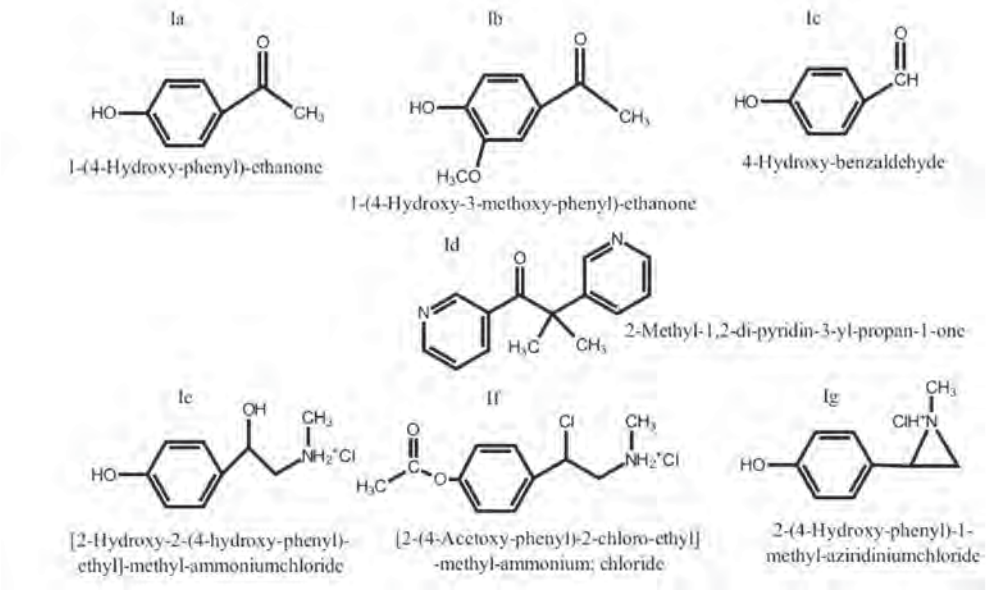
Two unrelated papers guided our investigation at this stage. In 1969 Williamson and O'Donnell [3] found that a bipyridil derivative, metyrapone (2-Methyl-1,2-di-pyridin-3-yl-propan-1-one, Id), inhibited the terminal step in mammalian glucocorticoid biosynthesis by binding to the cytochrome P450-dependent 11b-hydroxlase (P450c11) enzyme. And in 1973 Liggins and Fairclough published their elegant work on the initiation of parturition in sheep that showed that a surge in fetal cortisol was the primary trigger for the onset of birth in these animals [4]. The work attracted our attention as there was a structural similarity around the carbonyl moiety between metyrapone (Id) and the isolated acetophenones (Ia and Ib) and behavioural changes observed in rats during the feeding of the dried shrub as well as the acetophenones, were consistent with a block in corticosteroid production. Using the terminal enzyme in adrenal corticosteroidogenesis, cytochrome P450-dependent 11β-hydroxylase (CYP11B1), as bioassay an active fraction, S2, was prepared by methanol extraction, ultrafiltration liquid ion exchangers and reversed phase high performance liquid chromatography (HPLC). This fraction was, however, extremely labile which severely impeded the search for the structure of the active agent. Partial structure determination, using nuclear magnetic resonance spectroscopy (NMR) and fast atom bombardment mass spectrometry (FAB-MS) suggested the presence of synephrine and a highly reactive aziridine derivative [5].

A more stable analogue, 2-(4-acetoxyphenyl)2-chloro-N-methylethylammonium-chloride (CpdA) (1f), was subsequently synthesised. Like the active plant extracts, CpdA inhibited adrenal steroidogenesis and acted as a contraceptive. In addition, CpdA was stabilized by interaction with steroid binding globulins in plasma, thus enhancing biological activity *in vivo* [6]. The specific displacement of corticosteroids by CpdA prompted investigation into the potential of anti-inflammatory action of CpdA. It was found that CpdA acted as an anti-inflammatory agent by down-modulating TNF-induced pro-inflammatory gene expression [7].

Our work investigations of the shrub, *S. tuberculatiformis* Botsch, or the "Gannabos" as it is locally known, yielded Cpd A, a substance which evolved and later took on a life of its own. It turned out to be a most interesting

molecule, with potential anti inflammatory action and cancer preventing properties; a far cry from the prolonged gestation and contraceptive action initially investigated in the shrub. I believe the "Gannabos" still holds many secrets, some of which we will hopefully solve soon and others that will certainly keep more than one scientist occupied for years to come.

Keywords: compound A, cytochrome P450, steroid binding globulins, anti-inflammatory



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A Desert Plant-derived Compound against Inflammation

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Introduction: Glucocorticoid hormones (GCs) remain the mainstay for the treatment of various inflammatory disorders, because of their great efficacy. The long-term usage of GCs is, however, overshadowed by the occurrence of debilitating side-effects, like osteoporosis, skin and muscle atrophy, diabetes and neurological disorders. GCs exert their functions through binding to the glucocorticoid receptor (GR), a transcription factor that regulates gene transcription in a positive or negative way. Direct binding of activated GR in the promoter of target genes is believed to be the main pathway leading to metabolic gene expression (mainly hold responsible for the unwanted side-effects), whereas the interference of GR with the activity of other transcription factors, such as NF-κB or AP-1, greatly contributes to its desired anti-inflammatory capacities. 'Dissociated ligands' aim to separate GR-mediated transcriptional activation from transcriptional repression in order to achieve better side-effect profiles.
In this respect, a newly characterized, plant-derived, non-steroidal GR modulator, i.e. Compound A (CpdA), was tested both *in vitro* and *in vivo* for its dissociative effects.
Materials & Methods: We have used CpdA in several cellular *in vitro* assays as well as in *in vivo* disease models to test its dissociated properties, as compared to glucocorticoids.
Results: CpdA behaves as a potent (although weaker) anti-inflammatory agent, both *in vitro* and *in vivo*, as compared to the synthetic glucocorticoid Dexamethasone. However, as opposed to steroidal ligands, CpdA does not give rise to the gene-activating effects in cells, nor to increased blood glucose levels or hyperinsulinemia in the tested animals. Furthermore, CpdA can cross the blood-brain barrier and can thus also be used for the treatment of neurological disorders. As opposed to glucocorticoids, CpdA does not lead to GR desensitization or glucocorticoid unresponsiveness, nor to osteoporosis. Finally, CpdA differentiates between repression of NF-kB and AP-1 (the activity of which is actually slightly enhanced), and thus yields more beneficial effects, e.g. in case of skin inflammation.
Conclusion: It is possible to fully dissociate the gene-activating effects from the inhibitory actions of GR by imposing a monomeric structure to the receptor by so-called 'specific GR modulators' (SGRMs), like CpdA. Moreover, GR desensitization and osteoporosis can be avoided which adds to the beneficial effects for long-term treatments.

Anti-cancer Potential of Selective Glucocorticoid Receptor Activators: A Novel Approach to GR-targeted Chemotherapy

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Introduction: Glucocorticoid hormones are widely used for the treatment of patients with different T- and B-cell lymphomas and multiple myeloma. They are also used as an important component of combination chemotherapy of many epithelial cancers. Unfortunately, patients chronically treated with glucocorticoids develop numerous metabolic side effects. Thus, the development of selective glucocorticoid receptor (GR) activators (SEGRA) with improved therapeutic index is important.

GR regulates gene expression via (i) transactivation that requires GR homodimer binding to gene promoters, and (ii) transrepression mediated via negative GR interaction with other transcription factors, including major pro-inflammatory and pro-proliferative factors NF-kB and AP-1. It is well understood that GR transrepression plays an important role in therapeutic anti-inflammatory and anti-cancer effects of glucocorticoids. In contrast, many metabolic adverse effects are linked to GR transactivation. Thus, selective GR activators (SEGRA, also called "dissociated" GR ligands) that preferentially promote GR transrepression are expected to retain therapeutic properties of classical glucocorticoids causing fewer side effects.

Novel SEGRA Compound A (CpdA) is a synthetic analog of the aziridin precursor found in the African bush *Salsola Botch.* CpdA has unique ligand properties. Others and we showed that CpdA competes with glucocorticoids for GR binding, but prevents GR dimerization. Thus, it regulates only part of glucocorticoid-responsive genes, significantly shifting GR activity towards transrepression. CpdA is as effective as glucocorticoids in counteracting inflammation, but has fewer metabolic side effects related to glucose control, maintenance of hypothalamic–pituitary–adrenal axis, bone metabolism.

Objective: The anti-cancer effects of SEGRA including CpdA have not been studied. We explored anti-cancer potential of CpdA in different human epithelial and lymphoma cancer cells. As gene regulation by GR is cell type dependent, in parallel experiments we evaluated CpdA ligand properties and its effect on gene expression in cancer cells.

Results: We found that CpdA inhibited both growth and survival of highly malignant prostate cancer cells in GR-dependent fashion *in vitro*, and in xenograft model. We also tested CpdA anti-cancer activity in different transformed lymphoid cells expressing GR as well as in their counterparts with silenced GR, and showed that CpdA strongly suppressed growth and viability of human T-, B-lymphoma and multiple myeloma cells. Furthermore, primary leukemia cell cultures from the patients appeared to be equally sensitive to glucocorticoid Dexamethasone and CpdA.

It is known that GR expression is tightly controlled by ubiquitin-proteasome degradation system. We showed that pretreatment of prostate and lymphoid cancer cells with Bortezomib (BZ) – the first proteasome inhibitor approved by FDA for clinical use, resulted in significant accumulation of GR. We hypothesized that BZ augments CpdA effects as a selective GR modulator and enhances its chemotherapeutic activity. We indeed observed that BZ exerts potentiating effects on CpdA-mediated GR transrepression, and that BZ+CpdA do not efficiently induce GR transactivation. Further, the increased GR availability made cancer cells more sensitive to CpdA, and resulted in remarkable GR-dependent cooperation between CpdA and BZ in suppression of growth and survival of prostate cancer, lymphoma and multiple myeloma cells.

Importantly, CpdA+BZ differentially regulated GR-responsive genes. CpdA+BZ blocked activation of glucocorticoid-responsive pro-survival genes, but augmented BZ-induced endoplasmic reticulum stress via activation of ER stress down-stream pro-apoptotic gene CHOP/GADD153.

Conclusion: Our results indicate that pretreatment with proteasome inhibitors followed by selective GR modulators like CpdA could release the anti-cancer GR signaling at its maximal potential. This approach establishes a novel strategy for GR-targeted chemotherapy.

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Keywords: glucocorticoid receptor, selective GR activator (SEGRA), lymphoma, prostate cancer, proteasome inhibitor

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Molecular Mechanisms for Corticosteroids Resistance and Its Reversal

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Corticosteroids are the most effective anti-inflammatory treatment available and have been effective in the treatment of many inflammatory and immune diseases. However some patients and some inflammatory diseases respond poorly to corticosteroids[1]. Several molecular mechanisms have now been identified to account for the poor response to corticosteroids and therapeutic targeting of these mechanisms may therefore potentially reverse corticosteroids resistance. In patients with severe asthma several mechanism of corticosteroid resistance have been identified and these may require different therapeutic approaches, thus making it important to identify the mechanism of resistance. In chronic obstructive pulmonary disease (COPD), a common progressive inflammatory disease of the lung, there are no effective anti-inflammatory treatments and most patients are corticosteroid resistance. We have focused on the molecular mechanisms for corticosteroid resistance in COPD and how this may be reversed with existing and novel therapies.

Corticosteroids exert their anti-inflammatory effects through several molecular pathways, but one key mechanism is through switching off activated inflammatory genes via glucocorticoid-receptor (GR)-mediated recruitment of the nuclear enzyme histone deacetylase-2 (HDAC2) to the activated inflammatory gene complex to thus reverse the histone acetylation associated with gene activation. In addition, HDAC2 deacetylates acetylated GR allowing it to inhibit proinflammatory transcription factors, such as NF-κB [2]. In patients with COPD HDAC2 is markedly reducing in activity and expression [3] and this appears to be mainly a consequence of oxidative stress. HDAC2 is reduced by oxidative stress and nitric oxide, which form peroxynitrite that nitrates tyrosine residues in HDAC2, resulting in inactivation of its enzyme activity and to ubiquitination and subsequent proteasomal degradation. Oxidative stress also activates phosphoinisitide-3-kinase-δ (PI3Kδ), resulting in subsequent phosphorylation, ubiquitination and inactivation of HDAC2. Hypoxia also reduces HDAC2 expression via an inhibitory effect of HIF1α on HDAC2 gene transcription. Reduced HDAC2 expression may also account for the corticosteroid resistance seen in asthmatic patients who smoke and in some patients with severe asthma.

HDAC2 expression can be resorted by the use of a plasmid vector, resulting in restoration of corticosteroid responsiveness in COPD macrophages, thus demonstrating that corticosteroid resistance is potentially reversible. This reversal of corticosteroid resistance is also achieved by low concentrations of theophylline, which restores the low levels of HDAC2 levels in COPD macrophages to normal and reverses corticosteroid resistance *in vitro* and *in vivo* by selectively blocking oxidant-activated PI3Kδ. Clinical studies now show that low dose theophylline appears to restore corticosteroid responsiveness in COPD patients and larger clinical trials are currently underway. The tricyclic antidepressant nortriptyline and macrolide antibiotics work in a similar way by inhibiting PI3Kδ and these effects are mimicked by newly developed selective PI3Kδ inhibitors. The transcription factor Nrf2, which activates multiple antioxidant genes in response to oxidative stress is defective in COPD cells. The Nrf2 activator sulforaphane is effective in restoring Nrf2 activity and increasing HDAC2 to reverse corticosteroid resistance in COPD macrophages [4]. In the future treatments that reverse corticosteroid resistance may improve the management of COPD, severe asthma and in other inflammatory diseases, such as rheumatoid arthritis and inflammatory bowel disease, where corticosteroids are poorly effective in some patients.

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Epigenetic Alterations and Cancer Chemoprevention by Dietary Polyphenols

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The term 'epigenetics' refers to heritable changes in gene expression that are not attributable to permanent alterations in the DNA sequence. Three types of epigenetic changes that we currently best understand comprise of alterations in DNA methylation, histone modifications and non-coding RNAs including micro RNAs (miRNAs). Epigenetic events have also emerged as key drivers in cancer development, and a better understanding of these mechanisms is fundamental for our ability to successfully diagnose, treat, and prevent human cancer. The growing interest in cancer epigenetics stems from several factors. First, epigenetic changes are implicated virtually at every step of tumor development and progression. Second, epigenetic changes including DNA hypermethylation are an early event in tumor development, and may precede development of the *genetic* hallmarks of cancer. Third, in contrast to genetic changes, epigenetic alterations are potentially reversible; therefore, aberrant DNA methylation, histone acetylation and methylation provide an exciting opportunity for the development of novel strategies for cancer prevention. Fourth, recent studies have recognized that a cross-talk exists between various epigenetic processes. It is now apparent that DNA methylation and histone modification processes may cooperate to regulate gene expression. Similarly, expression of miRNAs may be regulated by DNA methylation. Collectively, this means that dietary intervention of even a single epigenetic process is likely to influence other epigenetic processes in a positive manner.

In view of this, not surprisingly, there is a substantial interest in the development of safe and effective inhibitors of DNA methyl transferases (DNMT) and histone deacetylases (HDAC). Of interest, there is increasing evidence that epigenetic changes are easily influenced by environmental, lifestyle and dietary factors, and some estimates suggest that over two-thirds of the cancer incidence can be accounted for by the environmental and dietary factors alone. Among all these factors, diet is probably the single most important factor which may influence carcinogenesis more comprehensively, because diet is readily modifiable and have the potential to modulate multiple epigenetic processes. Polyphenols in dietary botanicals represent a versatile group of phytochemicals with many potentially beneficial activities in terms of disease prevention. Dietary polyphenols (bioflavanoids) have antioxidant and anti-inflammatory properties that might explain their chemopreventive effects. However, the actual therapeutic potential of these compounds may not have been completely realized due to lack of understanding on the effects of these agents on epigenetic modifications. Recent, but limited evidence indicates that some of the polyphenols, including curcumin (from turmeric), genestein (from soy), EGCG (from green tea), diallyl disulfide (from garlic), sulforaphane (from broccoli) and resveratrol (from grapes) may induce epigenetic changes in various cancer cell lines. This presentation will describe some of the current literature on the role of the dietary polyphenols on epigenetic alterations, which will provide a strong scientific foundation for preclinical and human clinical intervention studies in future.

The Epigenetic Regulation of Autophagy in Cancer Cells and Its Impact on Chemotherapy: Role of Stroma and Inflammation

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In the present lecture, we present original findings regarding the involvement of autophagy in chemotherapy response in ovarian cancer, and the role of tumor microenvironment (i.e., infiltration of fibroblasts and macrophages type-2; inflammation, cytokines, hypoxia, oxidative stress) on its regulation.

Autophagy is a cellular catabolic process that contributes to cell and tissue homeostases through the regular elimination of damaged, aged and redundant molecules and organelles. Of the three know autophagy pathways, namely chaperon-mediated autophagy, microautophagy and macroautophagy (Figure 1), we will here consider only the latter. In macroautophagy (for simplicity, autophagy), protein macro-aggregates, large portions of membranes and entire organelles are entrapped within a double-membrane vesicle named the autophagosome, which is marked by the presence on both membranes of the lipidated isoform of LC3 II. Ubiquitinated substrates and mitochondria are specifically delivered to the autophagosome thanks to the binding to p62 or BNIP3. The autophagosome then reaches the microtubular organizing center where it encounters and fuses with lysosomes. Movement of organelles on microtubules depends on the activity of the Histone deacetylase HDAC6. Once the fusion has occurred, the lysosomal acid hydrolases (mainly Cathepsins D, B and L) degrade the autophagic substrates along with the external membrane proteins fo the autophagosome (including LC3 II). The fusion step is inhibited by drugs that rise up the vacuolar pH of endosomes and lysosomes, such as chloroquine, ammonium chloride and bafilomycin A. Under such conditions, the autophagy flux is interrupted and undigested substrates accumulate within autophagosomes. By degrading the cytoplasmic material, autophagy provides recycled monomeric substrates to the anabolic machinery. This function turns useful for cells experiencing nutrient shortage. Autophagy plays a complex role in the various phases of cancer development and progression. Because of its ability to remove all damaged and harmful molecules and organelles in the cell and to refill the synthetic machinery with recycled substrates, autophagy might either exert anti-cancer and pro-cancer activities. In fact, by promptly removing mutagenic pro-oxidants molecules, autophagy prevents cell transformation, thought it may turn in facilitating cancer progression by helping the cancer cell to overcome the lack of nutrients and of oxygen, as well as the attack of anti-neoplastic drugs that cause damage to proteins, DNA and organelles. The activation of autophagy requires an integer set of autophagy genes, including oncogenes and oncosuppressors, and of regulatory signaling pathways. Besides, autophagy is influenced by epigenetic factors, either intracellular (e.g. de-acetylation of proteins, microRNAs, reactive oxygen species) or extracellular (e.g., inflammatory cells, fibrobalsts, vascularization and cytokines).

Here, we will discuss the genetic and epigenetic factors that influence autophagy, and its potential involvement in ovarian cancer progression and in the response to chemotherapeutic treatments. It is known that ovarian cancer cells release chemotactic cytokines and growth factors that recruit fibroblasts, endothelial cells and macrophages, which azin turn contribute with their own secretions to form a dynamic tumor microenvironment. Inflammatory-related proteins (e.g., TNFα, IL-6, IL-1β, Lysophosphatidic acid) abnormally present in the tumor context and associated with ovarian cancer progression can affect autophagy. We have experimentally addressed this issue both in *ex vivo* biopsies from ovarian cancer patients and in *'in vitro'* ovarian cancer cells cultivated in the presence of cytokines or Lysophosphatidic acid. In the biopsies (see Figure 2), we have assessed the presence of autophagic cells and of type 2 Macrophages and cancer associated fibroblasts, that notoriously infiltrate the tumor stroma. Type 2 Macrophages were labeled as CD68, CD14 and CD206 positive macrophages. Autophagic cells were recognized for the expression of the autophagy markers LC3 and beclin 1 (the latter is an oncosuppressor that interacts with PI3k class III to start the signals for the formation of the autophagosome). In the *'in vitro'* cell model we could manipulate genetically the expression of autophagy related genes, and this allowed us to better assess the involvement of autophagy in the cytotoxic response to chemotherapeutics in the presence of Lysophosphatidic acid, which has been reported to confer chemoresistance to ovarian cancers. Our data point out how the microenvironment and the cytokine network epigenetically affect autophagy in cancer cells, an aspect that should be taken into account when designing chemotherapeutic strategies that target autophagy.

Keywords: autophagy, ovarian cancer, chemoresistance, inflammation, cytokines

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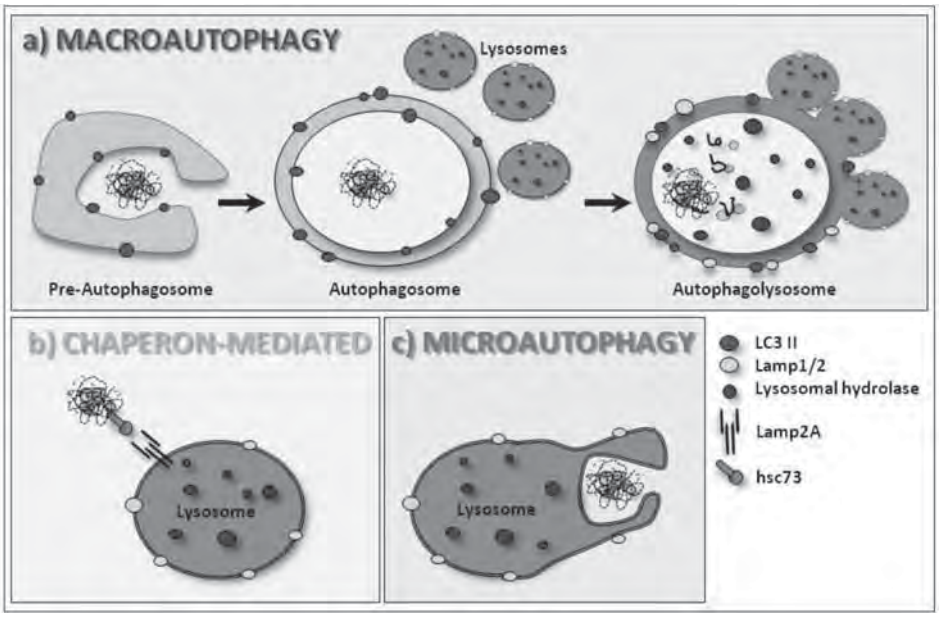

Figure 1. Scheme of the three autophagy pathways.

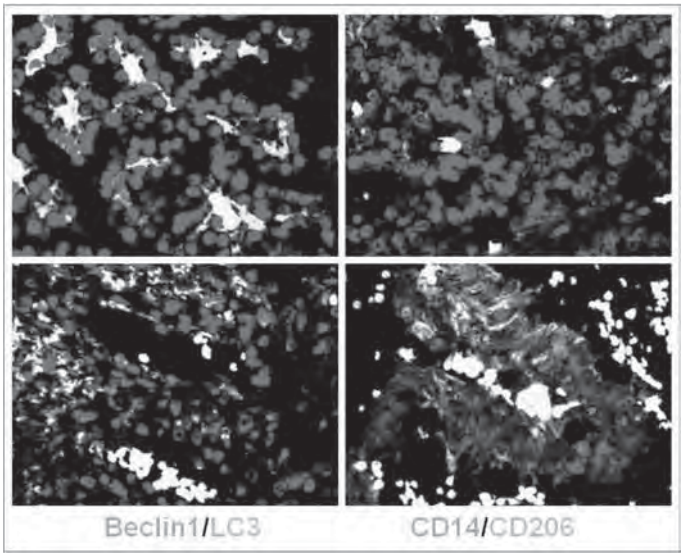

Figure 2. Immunofluorescence labeling of autophagy markers (LC3 and beclin 1) and of Type 2 Macrophages (CD14 and CD206) in ovarian cancer tissues.

The Consequences of Genome Wide Hypomethylation *in cis*

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Introduction: Loss of genome wide methylation levels at long interspersed element-1s (LINE-1s or L1s) in cancer commonly occurs [1] and causes gene expression changes and promotes genomic instability. However, the mechanisms of these two consequences were not well characterized. There are more than 500,000 LINE-1 copies in human genome. More than 10,000 LINE-1s are consist of contains 5 untranslated region, a target for DNA methylation. The cancer associated global hypomethylation mechanism causes DNA methylation lost in a generalized and locus specific fashion [2]. There are several mechanisms that alter gene expression and genomic integrity both *in trans* and *in cis*.
Objective: To report how we evaluated mechanisms for how LINE-1 hypomethylation in cancer regulates gene expression and promotes genomic instability *in cis*. For gene expression, we evaluated if cancer associated epigenetic modification of intragenic LINE-1s alter host genes expression [3]. For genomic instability, we explored the association between methylation and repair of endogenous DNA double strand breaks (EDSBs) [4, 5].
Materials & Methods: To evaluate the association between intragenic LINE-1s and expression of host genes, we used computational biology approach. The locations and sequences of LINE-1s containing 5'UTR were from L1base (http://l1base.molgen.mpg.de). Interestingly, transcriptional activity and CpG dinucleotide sites for methylation of intragenic LINE-1s are more conserved than intergenic LINE-1s. The expression statuses of genes containing LINE-1s were analyzed from expression profiling by array of cancers, normal cell treated with demethylating agent and Argonaute 2 knocked down cells, available from Gene Expression Omnibus (GEO). GEO is a database repository of high throughput gene expression data, (http://www.ncbi.nlm.nih.gov/geo). To evaluate genomic instability, we established a novel technique to measure the extent of EDSBs and methylation at LINE-1 sequences nearby EDSBs. .
Results: A significant numbers of genes containing LINE-1s of both normal cells treated with demethylation agents and cancers were repressed. In contrast, genes containing LINE-1s were up-regulated when AGO2 protein was depleted. Finally, AGO2 bound to pre-mRNA between transcriptional start sites and LINE-1s. When we measure EDSBs, we found significant levels of EDSBs in all human cell types, both in replicating and non-replicating cells. We called the EDSBs in non-deviding cells as replication independent EDSBs (RIND-EDSBs). The RIND-EDSBs ubiquitously possess higher levels of methylation than the cellular genome. This is in contrast to lower methylation levels of γ-H2AX-bound DNA. Therefore, cells free from an immediate DNA damage response to methylated RIND-EDSBs. Moreover, we found a significant number of methylated RIND-EDSBs that are retained within heterochromatin. Finally, a RIND-EDSB methylation levels was increased when cells were depleted of ATM.
Discussion: There are mechanisms that genome wide hypomethylation causing gene expression changes and increasing rate of mutation *in cis*. Lower methylation of LINE-1s in cancer increase LINE-1 RNA. The intragenic LINE-1 RNA forms complex with pre-mRNA and AGO2. Consequently, genes containing LINE-1s are repressed. The loss of DNA methylation also alters mechanisms how RIND-EDSBs are repaired. Methylated RIND-EDSBs are retained in heterochromatin and precisely repaired by ATM dependent NHEJ pathway. In hypomethylated genome, RIND-EDSBs are repaired by more error-prone NHEJ mechanisms.
Conclusion: Global hypomethylation in cancer alters expression levels of many genes and promotes genomic instability. We reported a study described the mechanism that hypomethylated intragenic LINE-1s repress gene expression via RNA-induced silencing complex, or RISC [3]. We also described a mechanism of genomic instability via error prone EDSB repair [4, 5].

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Keywords: genome wide methylation, global hypomethylation, long interspersed element-1s, genomic instability, replication independent endogenous DNA double strand breaks

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Inflammation Signaling in Skin Carcinogenesis

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Introduction: Solar UV is the major etiology of human skin cancer. Solar UV-induced skin inflammation signaling plays a critical role in human skin cancer.
Objective: To use a mouse model and human skin tissue to study the role of inflammation in skin carcinogenesis
Materials & Methods:
1) Knockout mouse model
2) Human skin tissue
Results: Solar UV-induced inflammation signaling plays an important role in skin carcinogenesis.
Discussion and Conclusion:
1) Solar UV is a potent inducer for skin inflammation.
2) Inflammation signaling can be inhibited by certain kinase inhibitors.
3) Blocked inflammation signaling can be used for skin cancer prevention.

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Keywords: solar UV, skin cancer, inflammation signaling

Glucocorticoid Stress Hormones and Resilience to Brain Disease

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Introduction: Every threat to integrity and homeostasis of the organism, either real or imagined, activates the stress system which includes the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system. These threats or stressors, as diverse as tissue damage, infection, metabolic disturbance, fluid loss and neural disturbances, will trigger e.g. inflammatory, immune, metabolic, volume and behavioural responses, respectively. Irrespective their peripheral, central or environmental origin the brain will monitor both the threats and the physiological responses. For this purpose the stressful sensory information is perceived, processed and appraised in the socalled limbic brain, which includes the amygdala for emotion regulation, the hippocampus for labeling emotions in time, place and context of the event and parts of the frontal cortex for planning and decision making. The outcome of this appraisal process is relayed and integrated in the paraventricular nucleus of the hypothalamus where the neuroendocrine, autonomic and behavioural response to this ultimately psychological stressor is orchestrated, with the aim to cope with the stressor. In this lecture I will focus on the mode of action of the stress hormones cortisol (which is not present in rodents) and corticosterone, that are secreted by the adrenals as endproduct of the HPA axis.

Findings: Cortisol and corticosterone (glucocorticoids) are secreted in hourly pulses to synchronize daily activities and sleep-related events. Superimposed on this ultradian/circadian rhythm the hormones coordinate in response to the stressor, adaptation and allocation of energy resources needed for recovery. The hormones are best known for their anti-inflammatory and immune suppressive clinical efficacy, but they also have profound effects on emotion, cognition and motivation. Mineralocorticoids (aldosterone) are the regulators of the Na/K balance. Ever since Selye the mineralocorticoids such as aldosterone and deoxycorticosterone are known to be pro-inflammatory. In the brain mineralocorticoids also complement and oppose glucocorticoid effects. How is this possible?

To exert their action the hormones bind to mineralocorticoid receptors (MR) and glucocorticoid receptors (GR). In epithelial cells (kidney, colon, salivary glands) the MR is protected from cortisol because 11-βHSD-2 converts the steroid to bio-inactive cortisone, and responds therefore to aldosterone regulated by the renin-angiotensin system in control of the electrolyte balance. Yet, in non-epithelial cells in vessels, heart and brain an MR is expressed that is not protected from cortisol by 11-βHSD-2. Here the MR *sees* predominantly cortisol which circulates in a 1000 fold excess over aldosterone. GR has a 10 fold lower affinity for cortisol than MR. During the ultradian/circadian pulses and the stress response MR and GR are therefore differentially activated. The high affinity MR is first activated and promotes rapidly stress (inflammatory) reactions in vessels, heart or in glial cells (induced for instance by Alzheimer β-amyloid toxicity) in brain, while via GR cortisol prevents these initial reactions from overshooting and mediates in slower fashion the e.g. anti-inflammatory and other genomic effects of cortisol.

MR and GR are co-expressed most abundantly in the limbic brain, and affectappraisal, learning and memory processes. The MR mediates the immediate effects of cortisol important for the selection of an appropriate coping response and thus for onset of the stress reaction, while via GR the steroid regulates the management of later adaptive phases including recovery from the stressor and the storage of the experience in the memory in preparation for the future. The MR- and GR-mediated actions have in brain an enormous diversity depending on contextual, spatial and temporal cues, which is reflected in distinct patterns of responsive gene networks. The temporal aspect has gained an extra dimension because of the recent discovery that the very same MR and GR involved in genomic regulation also mediate rapid membrane-localised actions on brain excitability. These immediate effects are crucial for cognitive flexibility in coping responses with stress. The receptors occur in functional genetic variants and are modified epigenetically in expression by life experience with lifelong consequences for brain plasticity and behaviour. Particularly interesting is the functional MR haplotype 2 variant that is associated with optimism and protection against depression.

Discussion: the following questions will be addressed

- How does cortisol act in the stressed brain? What is the role of MR and GR?
- MR- and GR-mediated actions operate in complementary fashion to maintain homeostasis, resilience and health. The MR helps to organize initial defense reactions, while via GR recovery, adaptation and preparation for future events is promoted. Does this balance in MR:GR mediated effects explain how glucocorticoid action can change from protective to harmful, if dysregulated? What is the cause of dysregulation? What are the consequences?

- If imbalance in MR:GR interaction enhances vulnerability to disease, how can this principle be employed for novel treatment strategies?

Conclusion: There is now ample evidence suggesting that stress hormones, including cortisol, can modify the onset and progression of disease. Since all threats to homeostasis and integrity, whether environmental, peripheral or central, ultimately are evaluated in the limbic brain it is important to learn how stress hormones can protect health in integrated fashion in body, brain and mind. Hence, MR- and GR-responsive susceptibility pathways mediating the action of cortisol within the stress system itself present potential novel targets to promote resilience still present in the diseased brain.

Ackowledgements: Supported by the Royal Netherlands Academy of Sciences.

Keywords: stress, brain, cortisol, mineralocorticoid receptors, glucocorticoid receptors

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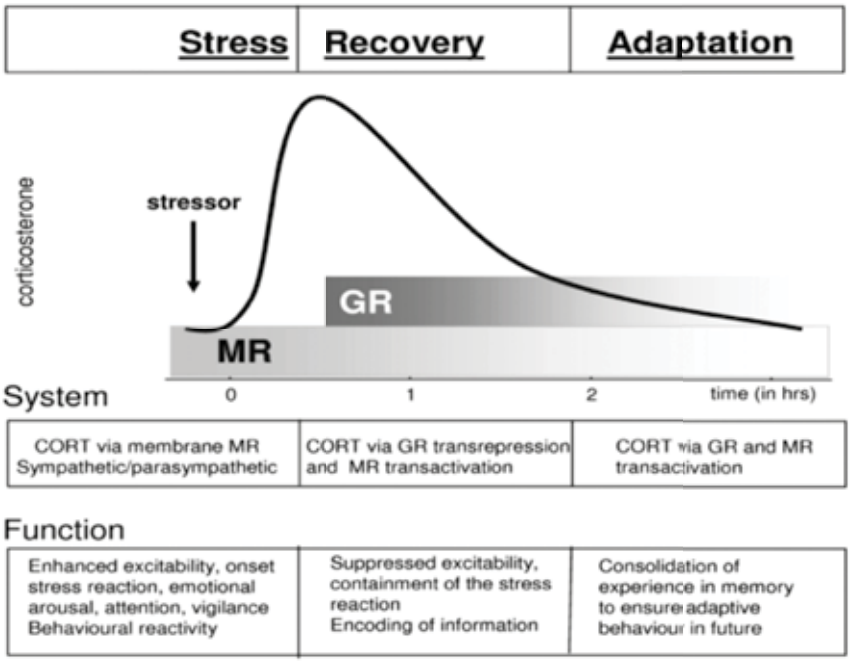

Figure: From de Kloet et al. 2005.

Functional Foods in Diet-induced Metabolic Syndrome

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The metabolic syndrome includes central obesity, insulin resistance, elevated blood pressure, impaired glucose tolerance and dyslipidaemia; these are accepted risk factors for cardiovascular disease and type 2 diabetes. The number of adults with metabolic syndrome is substantial and the prevalence is increasing in both developed and developing countries. The widespread occurrence of metabolic syndrome in humans means that there is an urgent need to study relevant causes and progression of the signs. These studies require viable animal models that adequately mimic all the aspects of the human disease, developing all major signs of metabolic syndrome, especially obesity, diabetes, dyslipidaemia, hypertension and possibly fatty liver disease and kidney dysfunction. Rodents have been used for many years as models of human disease, especially hypertension, diabetes and obesity. Our first aim was to develop a rat model of diet-induced metabolic, cardiovascular and liver changes that mimicked the human metabolic syndrome. Male Wistar rats were fed a high carbohydrate, high fat diet containing condensed milk (39.5%), beef tallow (20%), fructose (17.5%), rat food (15%) and minerals (1%) supplemented with fructose (25%) in the drinking water, for 16 weeks; condensed milk, beef tallow and fructose were replaced by corn starch in control rats.

During 16 weeks on this diet, rats showed progressive increases in body weight, energy intake, abdominal fat deposition and abdominal circumference along with impaired glucose tolerance, dyslipidaemia, hyperinsulinaemia and increased plasma leptin and malondialdehyde concentrations. Cardiovascular signs included increased systolic blood pressure and endothelial dysfunction together with inflammation, fibrosis, hypertrophy, increased stiffness and delayed repolarization in the left ventricle of the heart. The liver showed increased wet weight, fat deposition, inflammation and fibrosis with increased plasma activity of liver enzymes. The kidneys showed inflammation and fibrosis whereas the pancreas showed increased islet size. In comparison with other models of diabetes and obesity, this diet-induced model more closely mimics the changes observed in human metabolic syndrome.

Nutraceuticals and functional foods have been used for thousands of years to treat human disease, including the symptoms of the metabolic syndrome such as obesity, diabetes, hypertension and liver dysfunction. Our second aim was to determine whether these diet-induced symptoms could be reversed by supplementation with purple carrots, rutin, quercetin, caffeine, L-arginine or polyunsaturated fatty acids (ALA, EPA, DHA) from chia or fish oils as additions to the diet for 8 weeks starting 8 weeks after the diet was initiated.

Anthocyanins, phenolic acids and carotenoids are the predominant phytochemicals present in purple carrots. We compared the ability of purple carrot juice and β-carotene to reverse the structural and functional changes in rats fed the high carbohydrate, high fat diet. Purple carrot juice attenuated or reversed all changes while β-carotene did not reduce oxidative stress, cardiac stiffness or hepatic fat deposition. As the juice itself contained low concentrations of carotenoids, it is likely that the anthocyanins are responsible for the antioxidant and anti-inflammatory properties of purple carrot juice to improve glucose tolerance as well as normalising cardiovascular and hepatic structure and function.

Rutin, a flavonoid glycoside of quercetin, is present in many foods such as onions, apples, tea and red wine. In our high carbohydrate, high fat fed rats, rutin (approximately 100 mg/kg/day) and quercetin (approximately 50 mg/kg/day) reversed or prevented metabolic changes such as abdominal fat pads and glucose tolerance, reversed or prevented changes in hepatic and cardiovascular structure and function, reversed oxidative stress and inflammation in the liver and the heart, and normalised expression of liver markers. These results suggest a role for dietary rutin and quercetin in metabolic syndrome.

Treatment with caffeine in rats fed high carbohydrate, high fat diet decreased body fat and systolic blood pressure, improved glucose tolerance and insulin sensitivity, and attenuated cardiovascular and hepatic abnormalities, although the plasma lipid concentrations were further increased. Decreased total body fat, concurrent with increased plasma lipid concentrations, reflects the lipolytic effects of caffeine in adipocytes, likely owing to the caffeine antagonism of A1 adenosine receptors on adipocytes.

Chia oil contains the essential fatty acid, -linolenic acid. Compared to the high carbohydrate, high fat fed rats, chia oil-supplemented rats improved insulin sensitivity and glucose tolerance, reduced visceral adiposity, decreased hepatic steatosis, reduced cardiac and hepatic inflammation and fibrosis without changes in plasma lipids or blood pressure. Chia oil induced lipid redistribution with lipid trafficking away from the visceral fat and liver with increased accumulation in the heart. The stearoyl-CoA desaturase-1 products were depleted in the heart, liver and the adipose tissue of chia oil supplemented rats together with an increase in the substrate concentrations. The C18:1*trans*-7 was

preferentially stored in the adipose tissue; the relatively inert C18:1n-9 was stored in sensitive organs such as liver and heart and C18:2n-6, the parent fatty acid of the n-6 pathway, was preferentially metabolised. Thus, chia seeds and oil as a source of -linolenic acid induce lipid redistribution associated with cardioprotection and hepatoprotection. The fish oil fatty acids, EPA and DHA, produced different responses on fat redistribution, indicating that ALA is effective without conversion to the longer-chain fatty acids.

The interventions prevented inflammatory cell infiltration into the heart, liver and fat pads, and decreased plasma inflammatory biomarkers. These results strongly suggest that functional foods reverse the chronic low-grade inflammatory state that induces cardiovascular, metabolic and liver signs in this rat model of diet-induced metabolic syndrome.

The Protective Ability of Anatolian Plant Extracts against Glycoxidation and Oxidative Protein Damage

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"Glycoxidation" is a term used for glycation processes involving oxidation. Reactive carbonyl compounds (RCCs) formed during lipid peroxidation and sugar glycoxidation, namely Advanced lipid peroxidation end products (ALEs) and Advanced Glycation end products (AGEs), accumulate with ageing and oxidative stress related diseases, such as atherosclerosis, diabetes or neurodegenerative diseases. RCCs induce the "carbonyl stress" characterized by the formation of adducts and cross-links on proteins, which progressively leads to impaired protein function and damages in all tissues, and pathological consequences including cell dysfunction, inflammatory response and apoptosis. The prevention of carbonyl stress involves the use of free radical scavengers, antioxidants and cellular redox regulators. Carbonyl scavengers prevent carbonyl stress by inhibiting the formation of protein cross-links. We aimed to examine the signaling properties of AGEs/ALEs and AGEs/ALEs-precursors, their role in the pathogenesis of oxidative stress-associated diseases, and the effects of different herbal extracts and derivatives efficient in neutralizing AGEs/ALEs effects *in vitro* and *in vivo*. We compared the effects of an oleuropein reach (OLE-1) and a hydroxytyrosol rich (OLE-2) olive leaf extracts with the effects of standard compounds (quercetin, hydroxythyrosole and oleuropein) on HNE-induced toxicity in rat cardiomyocye (H9C2) cell cultures. Both extracts reduced HNE-toxicity, improved viability, attenuated ROS generation and protected $\Delta\Psi$(m). The effects of extracts on $\Delta\Psi$(m) was more than the individual effects of quercetin, oleuropein or hydroxytyrosole. SAPK/JNK and Hsp27-induced increase in the presence of HNE was inhibited especially by quercetin and other olive polyphenols. Olive polyphenols induced down-regulation of cl-caspase 3 and cl-PARP in cells under conditions of HNE-induced cellular stres. We also examined the effects of *Olea europea* L. (olive) leaf and fruit extracts and oleuropein on cytokine-induced or H_2O_2-induced β-cell toxicity. INS-1 cells incubated with olive extract showed a significant reduction in cytokine- and H_2O_2-induced ROS production, caspase 3/7 activation, and ameliorated abnormal antioxidant defense, mithocondrial function and insulin secretion. In other group of studies, pomegranate (*Punica granatum* L.) ethanolic seed and hull extracts were tested, in comparison with a commercial sample, for the inhibition of aldose reductase, an enzyme involved in the etiology of diabetic complications. Results showed that pomegranate extracts are presented as bifunctional agents combining aldose reductase inhibitory action with antioxidant activity and with potential therapeutic use in prevention of diabetic complications. On the other hand, the effect of *Punica granatum* L. seed oil extract (PSEO), rich in n-5 PUFAs) on activation of cultured BV-2 microglia was investigated. PSEO showed a moderately less inhibitory effect on LPS-stimulated NO- and TNFalpha-release and iNOS expression levels than standardized n-3 PUFAs mixture. However, unlike to stobadin and quercetin, only the PUFAs preparations effectively inhibited the apoptotic markers in microglia exposed to the toxic LPS concentration. Our data point to the first evidence of immunomodulation and cytoprotection of microglial cells by the pomegranate seed oil-derived n-5 PUFAs, indicating thus their neuroprotective efficiency comparable to one of n-3 PUFAs. We also demonstrated that quercetin efficiently affects the multiple key molecular mechanisms involved in the etiology of both age-related and diabetic cataract, namely oxidative stress, non-enzymatic glycation, polyol pathway, calpain protease action, and lens epithelial cell signaling. Anotolian plant extracts sharing antioxidant, aldose reductase inhibitiory activity and carbonyl scavenger properties represent a new therapeutic challenge in the treatment of carbonyl stress-associated diseases such as diabetes and its complications.

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Keywords: glycoxidation, carbonyl stress, protein oxidation, apoptosis, H9C2-cardiomyocytes, pancreatic INS-1 cells, BV-2 microglia, olive leaf and fruit extracts, pomegranate seed oil and fruit extracts, quercetin, oleuropein, hydroxytyrosole

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15-Hydroxyprostaglandin Dehydrogenase as a Novel Molecular Target for Chemoprevention of Inflammation-associated Carcinogenesis

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Overproduction of prostaglandin E2 (PGE_2), as a major product of cyclooxygenase-2 (COX-2), has been reported to be implicated in inflammation-associated carcinogenesis. The intracellular level of PGE_2 is regulated not only by its biosynthesis, but also by the degradation process. 15-Hydroxyprostaglandin dehydrogenase (15-PGDH) is the key enzyme that catalyzes the first step in the inactivation of PGE_2. 15-PGDH has been known to be as a physiological antagonist of COX-2. Overexpression of COX-2 and repression of 15-PGDH may coordinately increase levels of PGE_2 in the tumor microenvironment, and exacerbates the carcinogenic process. However, the molecular mechanism underlying interaction between COX-2 and 15-PGDH remains largely unknown. In the present study, we observed that expression and activity of 15-PGDH were decreased in the colonic mucosa of dextran sodium sulfate (DSS)-treated mice, while the levels of COX-2 was elevated. To determine whether 15-PGDH is negatively regulated by COX-2, we utilized a selective COX-2 inhibitor celecoxib. Oral administration of celecoxib increased the 15-PGDH expression while the same treatment decreased COX-2 expression in DSS-treated mouse colon. Moreover, 15-PGDH expression in colonic mucosa following treatment with azoxymethaneplus DSS was more prominent in COX-2 knockout mice than that observed in COX-2 wild type animals. Likewise, levels of constitutively expressed 15-PGDH were higher in COX-2 deficient mice. We also observed that the mRNA levels and catalytic activity of 15-PGDH were upregulated by knock down of COX-2 using designed siRNA in colon cancer cell lines. In patients with colon tumors, the expression of 15-PGDH was markedly reduced in adenomas and carcinomas, compared with that in normal surrounding tissues. These finding suggest that expression of 15-PGDH is negatively regulated by COX-2, which may contribute to the inflammation-associated colon carcinogenesis.

Some peroxisome proliferator-activated receptor-γ ligands, non-steroidal anti-inflammatory drugs, and histone deacetylase (HDAC) inhibitors exert their chemopreventive and chemotherapeutic effects by inducing 15-PGDH expression while blocking COX-2. We have also found that curcumin, a yellow colouring agent present in the rhizome of *Curcuma longa* Linn (Zingiberaceae), induces expression of 15-PGDH in normal rat gastric mucosa cells (RGM-1) in concentration and time-dependent manners. The mRNA level of 15-PGDH was also increased by curcumin treatment. By using deletion constructs of 15-PGDH promoter, we found that activator protein-1 (AP-1) is the most essential transcription factor responsible for curcumin-induced upregulation of 15-PGDH expression. Curcumin enhanced the expression of c-Jun, c-fos and CREB in the nuclear fraction of RGM-1 cells. In contrast, tetrahydrocurcumin which lacks the α,β-unsaturated carbonyl group failed to induce expression 15-PGDH, suggesting that the electrophilic α,β-unsaturated carbonyl group of curcuminis essential for induction of 15-PGDH expression by curcumin. In this context, 15-PGDH may represent a novel molecular target of anti-inflammatory chemopreventive agents.

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Keywords: inflammation, chemoprevention, phytochemicals, 15-PGDH

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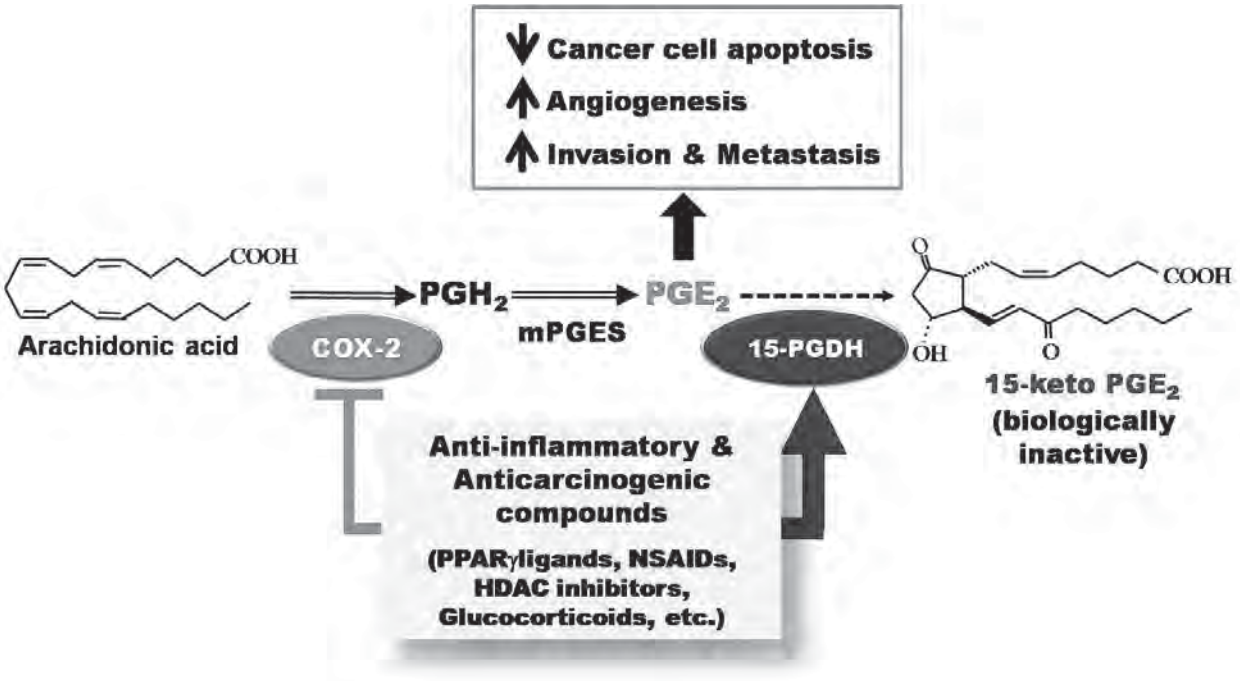

Figure: Induction of 15-PGDH expression may contribute to anti-inflammatory and anti-carcinogenic activities of chemopreventive agents

The Role of Biotechnology in Preserving and Studying Anti Cancer and Anti Inflammatory Activities of Some Syrian Medicinal Plants

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Medicinal plants have been used in virtually all cultures as a source of folkloristic medicine and often represented the original sources of most drugs. Syria has a great diversity of wild and cultivated plants due to the varied geography and climate. It has been estimated that more than 3500 species of vascular plants grow in Syria. The medicinal plants, comprising a good proportion of Syrian flora, are directly collected by rural population to prepare traditional medicines.

Medicinal plants in the Mediterranean regions are becoming increasingly rare due to both ongoing destruction of their natural habit, as well as over harvesting of wild species, the influencing factors of modernization; i.e., urbanization, migrations, detrimental climatic and environmental changes.

The biotechnology play a major role not only in preserving medicinal plants and wild species from deterioration, but also very useful for studying the biological activities of medicinal plants and their potential use as anti-inflammatory and anti tumorigenic compounds.

In my speech, I am going to talk on following points:

1-The use of plant tissue culture techniques very useful to multiply and conserve some medicinal plants from the deterioration, examples *Thymus syriacus* and wild iris (Black iris and *Iris aurantica*). Study of the effect of callus culture of Juniper on the growth of pathogenic and cancerous cells was investigated.

2-The bioactivity of the crude extracts of some medicinal plants to evaluate their effects on pathogenic bacteria belonging to Gram-positive and Gram-negative species (example *Juniperus excelsa*).

3-Study and combine up-to-date ethnobotanical knowledge with modern molecular biology, using reporter gene assays and their signaling pathways as molecular target, in order to identify potential anti-cancer natural products from some Syrian medicinal plants (examples *Thymus syriacus, Arcatium lapa, Juniperus excelsa*….).

4-Identification and chemical analysis of active extracts in order to identify active principles compounds by HPLC and GC-MS. (examples *Juniperus excelsa* and *Wild iris*).

To our best knowledge, this is the first report on the anticancer activities of crud extracts of *Juniperus excelsa*, *Thymus syriacus*, and *Arcatium lapa*, using NF-κB-driven reporter gene assays and MTT citotoxicity assays (In this report, we show inhibitory results of methanolic extracts of leaves and berries of *Juniperus excelsa* and *Thymus syriacus*, *Arcatium lapa*, on the growth of cancerous cells), identification and estimation of proanthocyanidins (catechin, epicatechin, dimeric procyanidins, and other oligomeric procyanidins) and the flavonoids such as Quercitin are likely the active phytochemicals in leaf and berries of *Juniper*. Further studies will be required to understand structural diversity of Pas and flavonoids in Junipers.

Poster and Oral Presentations

A:	Molecular Mechanisms
B:	Cancer Detection and Treatment
C:	Chemoprevention by Natural Compounds
D:	Glucocorticoid Receptors
E:	Epigenetics

Para-Phenylenediamine Induces Apoptosis via Activating MAPK Signalling Pathway and Inhibiting PTK-Ras-Raf-JNK Survival Pathway in NRK-52E Cells

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Introduction: *Para*-phenylenediamine (*p*-PD), a suspected carcinogen and was widely used in two-thirds of the hair dye formulations marketed. It is worthwhile to study the toxicology of *p*-PD causes it is a global health issue.
Objective: We elucidate the role of the mitogen-activated protein kinase signaling pathway, as well as the survival-associated PTR-Ras-Raf-JNK pathway on the growth of NRK-52E cells.
Materials & Methods: The cell viability was evaluated by trypan blue exclusion assay. The cell death due to apoptosis was determined by cell cycle analysis and Hoechst 33258 staining. The effects of *p*-PD on the proteins expression were performed by Western blot.
Results: Our results showed that *p*-PD caused apoptotic changes in cell morphology and a decrease in cell viability in a dose-dependent manner. Cell cycle analysis later confirmed that cell death was due to apoptosis, sub-G1 phase increased in a dose-dependent manner. Treated cells also exhibited nuclear damage when stained with Hoechst 33258 dye. We also examined the effects of the *p*-PD on the signalling pathways by Western blot. *p*-PD treated cells up-regulation of phospho-SAPK-JNK and phospho-p38 proteins expression. However, down-regulation of Ras, Raf and phospho-ERK proteins expression.
Conclusion: *p*-PD induced NRK-52E cells apoptosis, which is via MAPK and PTK-Ras-Raf-JNK signalling pathways.

Keywords: *para*-phenylenediamine, apoptosis, NRK-52E cells

Roles of Spleen Tyrosine Kinase in IL17-induecd CCL20 Chemokine Expression in Keratinocytes

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Introduction: IL-17A plays an important role of autoimmune diseases such as psoriasis. It has strongly revealed the clinical inhibition of IL-17A for psoriasis. Syk is a non-receptor tyrosine kinase and has been implicated as a critical mediator in various immune stimulation.
Objective: To investigate the role of Syk in IL-17A signaling.
Materials & Methods: Using by IL-17A-stimulated expression of CCL20 in normal human epidermal keratinocytes as a cell model to study the role of Syk in this aspect.
Results: IL-17A induces CCL20 gene and protein expression in time- and concentration-dependent manners. The activation of IKK, NF-B, JNK and Syk were observed during IL-17A stimulation. By Syk siRNA and TAK inhibitor, we found that Syk is an upstream signal molecule of TAK. Inhibition of Syk attenuated all signal kinases activation and CCL20 secretion induced by IL-17A. Data of reporter assay showed that IL-17A-elicited CCL20 upregulation is depending on Syk-mediated NF-B pathway. Using immunoprecipitation also indicated the interaction of Syk with TRAF6 and Act1 under IL-17A stimulation. However, the interaction of TRAF6 and Act1 under IL-17A stimulation was diminished when Syk expression was repressed by Syk siRNA approach.
Conclusion: We identify Syk as an upstream signaling regulator in IL-17R-mediated Act1-TRAF6 interaction, and demonstrate that Syk plays an essential role for IL-17R-stimulated NF-B activation and CCL20 gene transcription in human keratinocytes. All these findings not only unmask a new role of Syk in IL-17A-mediated inflammatory response, but also shed a new light into the potential therapeutic target of Syk in psoriasis.

Keywords: Syk, psoriasis, IL-17A, CCL20

Study of Interactions between CKIα and APC in Intestinal Homeostasis and Disease

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Introduction: The Wnt signaling pathway controls gut homeostasis and its deregulation results in tumorigenesis. Indeed, Wnt pathway mutations are frequent in colorectal cancer, mostly in APC, the prominent negative regulator of Wnt signaling. However, whereas gut APC inactivation in model mice leads to cancer, homeostasis is maintained following conditional ablation of CKIα, another Wnt antagonist, due to concomitant induction of p53.
Objective: We aim at inducing p53 activation via CKIα loss to prevent and treat APC-mutated intestinal tumors.
Materials & Methods: The $APC^{+/min}$ mouse model harbors a heterozygous germline mutation in APC and following LOH, develops vast number of adenomatous polyps in the small bowel. We crossed the $APC^{+/min}$ mice onto gut-specific CKIα knockout mice to invoke a p53 response in APC-mutated tumors.
Results: CKI loss triggered a p53 response throughout the small bowel of $APC^{+/min}$ mice, yet tumor numbers were not reduced. However, the growing tumors evaded CKI deletion, suggesting a selection against CKIα loss in APC mutated enterocytes.
Conclusion: Our data indicates synthetic lethality ofCKIα and APC loss. CKIα seems obligatory for the survival of benign tumors, and while its loss in a wild-type intestine is bearable, it is deleterious with APC mutation. It thus might be of interest to develop new therapeutics based on CKIα inhibition for colorectal cancer.

Keywords: Wnt pathway, APC mutation, CKIα inhibition, p53 activation, synthetic lethality

Hodgkin and Reed-Sternberg Cells Secrete Soluble Factors to Modulate Endothelial Cell-T Cell Interactions in Classical Hodgkin Lymphoma

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Introduction: Classical Hodgkin Lymphoma (CHL) is characterized by the presence of a small minority of malignant Hodgkin and Reed-Sternberg cells (H-RS cells) surrounded by a massive mixed of inflammatory infiltrates. $CD4^+$ T helper 2 cells, regulatory T cells and $CD8^+$ cytotoxic T cells form a significant part of this infiltrates. The mechanisms underlying T cell recruitment into the involved lymph nodes are still unknown.
Objective: The aim of this study is to elucidate whether H-RS cells can modulate endothelial cell-T cell interactions in CHL.
Materials & Methods: KM-H2 cell is used as a representative H-RS cell line. Adhesion molecules expression on the endothelial cells (EC) is studied by cell based ELISA and the mechanism involved is studied by Western blot.
Results: ELISA analyses show that KM-H2 C/S-stimulated EC up-regulate ICAM-1, VCAM-1 and E-selectin expression. C/S harvested from JNK inhibitor and COX activity treated KM-H2 show less stimulatory activity. ELISA analysis shows reduced ICAM-1, VCAM-1 and E-selectin expression on inhibitors treated KM-H2 C/S stimulated EC. Western blot analysis show that treatment of KM-H2 cells with JNK inhibitor reduced expression of phosphorylated JNK and C-Jun protein. Level of phosphorylated C-Fos and total C-Fos remained unchanged in JNK inhibitor treated KM-H2 cells. Level of phosphorylated C-Fos and total C-Fos reduced significantly in COX inhibitor treated KM-H2 cells.
Conclusion: Our data suggest that in CHL, malignant H-RS cells secrete soluble mediators which can modulate EC function. JNK and COX pathways are involved in regulating the production of soluble mediators from KM-H2 cells.

Keywords: T cell recruitment, modulate endothelial cell function, JNK, COX

Mesenchymal Stem Cells within Gliomastroma Promote Cancer Progression

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Introduction: A variety of stromal cells in tumor microenvironment enhance growth of the primary tumor as well as facilitate its metastatic dissemination to distant organs. In line with this, mesenchymal stem cells (MSCs) have been recently described to reside in tumor-associated stroma. However, the involvement of mesenchymal stem cells in brain tumor pathophysiology has not been addressed.
Objective: We aimed to understand the functional role of MSCs to glioma malignancy in tumor microenvironment.
Materials & Methods: MSCs were isolated from bone-marrow (BM) and glioma specimens as an independent cohort. The identity of MSCs was confirmed by FACS analysis. The cells were multipotent and positive for mesenchymal markers (CD105, CD90, CD73) and negative for endothelial (CD31), pericyte (NG2) surface markers. Migration or invasion assays were done in Trans-well that was pre-coated with matrigel or not. Cytokine array (R&D system) and RT-PCR were performed.
Results: Glioma cells acquired migratory, invasive traits along with mesenchymal phenotypes when co-cultured with glioma-associated MSCs. Notably, we found that glioma-associated MSCs promotes glioma invasiveness by secretion of cytokines C5α, GROα that were not secreted by BM-MSCs. In addition, glioma-associated MSCs promoted differentiation of glioma stem cells (GSCs) inducing the expression of neuronal (Tuj1), astrocyte (GFAP) and oligodendrocyte (Olig2) markers. Although BM-MSCs also promoted differentiation of GSCs, BM-MSCs did not induce invasive trait in glioma cells.
Conclusion: Collectively, our study suggest that glioma-associated MSCs, but not BM-MSCs, contribute to tumor microenvironment through the secretion of C5α and GROα, facilitating glioma cells to more malignant phenotypes.

Keywords: glioma, mesenchymal stem cell, tumor-microenvironment

Protein Kinase C δ Supports the Maintenance of Stemness and Malignancy in Glioma Cells

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Introduction: Protein kinase C (PKC), serine/threonine kinases represent key components of signal transduction pathways that regulate proliferation, cell survival. Previous studies have shown that the levels of PKC activity are much higher in glioma than in the normal brain. In addition, high levels of PKC activity are positively correlated with malignant phenotype of gliomas. However, the molecular mechanisms by which PKC contributes to these processes are not clearly understood.
Objective: In this study, we aimed to investigate the role of PKC in the maintenance of glioma stem cells (GSCs).
Materials & Methods: PKCδ expression was modulated by small interference or short hairpin RNA in glioma cells U87, U373 and patient-derived glioma X01 cells. GSC population was identified by stemness-related gene expression, clone-forming ability at single cell levels, tumorigenic capacity in soft-agar colony formation and orthotropic implantation in mouse brain.
Results: We found that PKCδ is preferentially activated in GSC population. Knockdown of PKCδ decreased the expression of GSC markers and sphere-forming ability. Also, down-regulation of PKCδ suppressed tumorigenic potential both *in vitro* and *in vivo*. Notably, PKCδ activation promoted the secretion of inflammatory cytokines, Interleukin (IL)-6 and IL-23 through the activation of STAT3. The PKCδ-mediated secretion of cytokines IL-6 and IL-23 was promoted by the signaling axis, JAK-PKCδ-STAT3 and JAK-PKCδ-AKT, implicating the existence of positive feedback loop.
Conclusion: Taken together, this study suggests that GSCs make use of positive feedback loop to activate the signaling axis; JAK-PKCδ-STAT3 and JAK-PKCδ-AKT, thereby maintaining their own population and malignancy in glioma.

Keywords: PKCδ, glioma stem cells, interleukin (IL)-6, IL-23, JAK- STAT3

The Relationship between SETD2, JMJD2C and TIP60 mRNA Expression Levels and Non Small Cell Lung Carcinoma

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Introduction: Lung cancer is the most common cause of death due to cancer in both men and women throughout the world. Alterations in epigenetic pathways have been shown to be implicated in common human diseases. Histone modifications are controlled by histone modifying enzymes.
Objective: Aim of this study is to identify the relationship between mRNA expression of histone modifying genes (SETD2, TIP60, JMJD2C) and development of NSCLC. In this study, the expression profiles of three histone modifying genes of cancer tissue and noncancerous part from 44 patients of non small cell lung cancer were investigated.
Materials & Methods: All of 44 patients had a diagnose of NSCLC. During operation, samples from tumor and the normal lung tissue were taken. Total RNA was isolated from tissue samples. Histopathologic confirmation of tissues were made by pathology department. The mRNA expression levels were measured by qPCR. Glyceraldehyde-3-phosphate dehydrogenase gene was used as the housekeeping gene. Our study was approved by local ethical committee.
Results: When the mRNA expression levels of these genes in lung cancer tissues were compared with those of normal lung tissues, our findings revealed 1.62 and 1.95 fold decrease in TIP60 and SETD2 expressions on mRNA level and these results were statistically significant ($p<0.05$). For JMJD2C a 1.7 fold increase in malign tissues was found but this lightly increase was not significant ($p=0.18$).
Conclusion: Our findings indicate a possible association of SETD2 and TIP60 genes mRNA expression with the pathogenesis of NSCLC. To confirm these findings, further molecular investigation is needed.

Keywords: non small cell lung carcinoma, SETD2, TIP60, JMJD2C, expression

Tumor Suppressive ADAM15 Exosomes Released from Human Macrophage

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Introduction: Human macrophages protect the bodies from foreign microorganisms and tumor in immune response.
Objective: In this study, we investigated the role of ADAM15 exosomes released from human macrophages.
Materials & Methods: Human primary monocytes were isolated from human blood using a monocyte isolation kit. Exosomes were isolated from monocytes with or without LPS stimulation by sequential centrifugation and filtration. Functional effects of exosomal ADAM15-derived macrophage were examined by using cell proliferation assay and migration assay.
Results: The present study shows that human macrophages, derived from human monocytes, not only express a disintegrin and metalloproteinase (ADAM) 15 proteins on the cell surface and but also release ADAM15 into the extracellular space as an exosome component. The releases of ADAM15 exosomes from macrophages are significantly elevatedby lipopolysaccharide (LPS) stimulation, indicating that ADAM15 release is stimulated by macrophages activation. The ADAM15 exosomes derived from human macrophages strongly suppress cancer proliferation and migration, indicating an anti-tumoric activity of exosomes. Notably, the macrophage-derived exosomes increase macrophage proliferation and migration itself. The ADAM15 exosomes suppress the tumor growth and prolong the survival of the mice bearing tumors in nude mice model.
Conclusion: This suggests the functional significance of macrophages exosomal ADAM protein in tumor suppression.

Keywords: ADAM15, exosomes, macrophage

Identification and Tumor-suppressive Function of ADAM15 as an Exosomal Component

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Introduction: A disintegrin and metalloproteinase (ADAM) 15, a widely expressed human membrane protein, is the only ADAM protein containing an Arg-Gly-Asp (RGD) motif in its disintegrin-like domain.
Objective: The aim of this study is to identify ADAM15 as an exosomal component and to investigate the functional mechanism of exosomal ADAM15.
Materials & Methods: Exosomes were isolated from tumor-conditioned medium by sequential centrifugation and 0.22μm-pore filtration. Exosomal ADAM15 was verified by sucrose density gradient fractionation, immunoelectron-microscopy, and flow cytometry analysis. The function of exosomal ADAM15 was investigated using immunoblotting, exosome-integrin binding assay, cell migration assay, and Xenograft tumorigenesis assay.
Results: We show that ADAM15 is released into the extracellular space as an exosome component, and that transfer of ADAM15 from the plasma membrane to the exosome is greatly enhanced by phorbol 12-myristate 13-acetate. ADAM15-rich exosomes exhibit improved binding affinity for integrin $\alpha v\beta 3$ and thereby interfere with the binding of integrin v3 to vitronectin. The exosomes significantly suppress cell adhesion, growth, and migration on vitronectin and fibronectinin *vitro*, as well as *in vivo* tumor growth. Experimental evidences indicate that RGD motif in the disintegrin-like domain of ADAM15 is the structural element which is responsible for the suppressive effects on the tumor growth.
Conclusion: This work suggests the ADAM15 exosomes-mediated regulatory mechanism on tumor growth.

Keywords: ADAM15, exosomes, tumor suppression

Function and Mechanism Study of a Novel Histone Deacetylase Inhibitor YH508 in Rheumatoid Arthritis Synovial Fibroblasts and Experimental Arthritis Model

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Introduction: The pathology of rheumatoid arthritis (RA) includes synoviocytes proliferation, expression of inflammatory mediators, and persistent recruitment of immune cell, and these might result from dysregulation of epigenetic gene control. Since HDAC expression profile in RA synovial tissue was different from that of normal control, there is a prospect of developing new therapies with HDAC inhibitor.
Objective: To examine the anti-rheumatic effect of YH508, a histone deacetylse (HDAC) inhibitor, by *in vitro* and *in vivo* model.
Materials & Methods: HDACs protein expression was determined by Western blot. Cytokines production was studied by ELISA. Osteoclastogenesis was evaluated by TRAP stain and flow cytometry. *In vivo* study was determined by Adjuvant Induced Arthritis rat model and IHC stain.
Results: The IC50 values of YH508 against enzymatic activity of HDAC1, 2, 3, 6, and 8 were 4, 5, 1, 8, 22 nM, respectively; all of which were significantly lower than SAHA, indicating that YH508 is more potent than SAHA. Treatment of RAW264.7 macrophages and RA synovial fibroblasts with YH508 dose-dependently inhibited cytokines (IL-6, nitric oxide, PGE2) secretion. In addition, YH508 dramatically suppressed RANKL/M-CSF (50/50 ng/mL) induced osteoclastogenesis at low concentration. The *in vivo* anti-arthritic effects of YH508 were evaluated in a *Mycobacterium butyricum*-induced arthritis model in rats, YH508 (25 mg/kg) significantly inhibited paw swelling, bone destruction and reduced serum cytokine levels.
Conclusion: Our results demonstrate that HDAC inhibitor YH508 inhibits the development of arthritis, suggesting that it might provide a new therapeutic approach to inflammatory arthritis diseases.

Keywords: histone deacetylase inhibitor, rheumatoid arthritis, epigenetics

Autophagy Inhibition Regulates the Expression of Pro-inflammatory Cytokines and Interferon-β in Macrophages

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Introduction: Autophagy intersected with innate immune responses and link to the elimination of intracellular pathogens. TLRs are autophagy sensors and can induce autophagy to control the replication of pathogens. However, the role of autophagy in macrophage-mediated inflammatory responses remains elusive.
Objective: Using a pharmacological inhibitor of autophagy (3-methyladenine, 3-MA) and ATG5 siRNA, we aim to get a thorough understanding on the influence of autophagy on TLR4-induced inflammatory responses in murine macrophages.
Materials & Methods: We used RAW264.7 macrophages in our study. The cell viability was determined by MTT assay, the gene expression was determined by real-time RT-PCR. The siRNA for ATG5 and GSK3β were transfected by lipofectamine. The tandem fluorescent-tagged LC3 construct (tfLC3) was used to detect autophagosome and autolysosome by fluorescent microscope.
Results: We found 3-MA could enhance LPS-induced NF-κB activation and production of TNF-α, iNOS, COX-2, IL-1β, and IL-12β. In contrast, 3-MA suppressed LPS-induced IFN-β production and STAT signaling. 3-MA can, through inhibition of Akt as a result of class I PI3K interference, positively regulate p38, JNK, and p65, but negatively regulate TBK1 and IRF3. We found 3-MA-induced effects were reversed by either GSK3β inhibitors or si-GSK3β but not affected in LPS-stimulated macrophages with si-ATG5 treatment.
Conclusion: Our results not only shed new lights on the action mechanisms of 3-MA to differentially regulate inflammatory outcomes derived from TLR4-mediated MyD88 and TRIF pathways, but also highlight the necessity to check autophagy status upon taking 3-MA as a general autophagy inhibitor.

Keywords: 3-MA, Akt, GSK3β, IRF3, inflammation

Role of Bacterial Endotoxin in Toll-like Receptor 4-mediated Growth of Endometriosis

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Introduction: Endometriosis is an estrogen-dependent chronic inflammatory disease. The exact pathogenesis or physiopathology of endometriosis is still debatable. Aside from ovarian steroids and secondary inflammatory mediators, information regarding the effect of initial inflammatory mediator on endometriosis is limited.
Objective: We investigated the role of bacterial endotoxin (LPS) in women with and without endometriosis.
Materials & Methods: Peritoneal fluid (PF) was collected from 58 women with endometriosis and 28 control women during laparoscopy. Menstrual blood (MB) was collected from a proportion of these women. Macrophages from PF and eutopic/ectopic endometrial cells were isolated in primary culture. Expression of Toll-like receptor 4 (TLR4) was examined at gene and protein levels. Using limulus amoebocyte lysate test, endotoxin (LPS) level was measured in menstrual fluid (MF) and PF. Cell proliferation effect was examined by bromodeoxyuridine (BrdU) incorporation assay. MB was cultured for the isolation of *Escherichia coli (E. coli)*.
Results: Colony formation of *E. coli* was significantly higher in the MB of women with endometriosis (10^5-10^7 CFU/ml) than in control women ($<10^2$ CFU/ml). *E. coli*-derived endotoxin levels in MF and PF were significantly higher in women with endometriosis than in controls. The production of a number of macromolecules by LPS-treated macrophages was significantly higher in women with endometriosis than in controls. Pre-treatment of cells with anti-TLR4 antibody abrogated LPS-stimulated secretion of macromolecules as well as LPS-promoted growth of eutopic and ectopic endometrial cells.
Conclusion: A substantial amount of endotoxin in MF and PF is involved in pelvic inflammation and may promote TLR4-mediated growth of endometriosis.

Keywords: endometriosis, endotoxin, TLR4, innate immunity

Pax4 Double Variants, *R192H* and *P321H*, Impaired Survival of Pancreatic β-cells Cultured in High Glucose Medium

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Introduction: *Pax4 R192H* and *P321H*, was associated with type 2 diabetes in Thai population.
Objective: This study examined transcriptional repression activity of *Pax4* double variants on its target promoters. Also, the function of *Pax4* on the proliferation of pancreatic β-cells cultured in high glucose medium, the condition that induces pancreatic β-cell death via increased oxidative and endoplasmic reticulum (ER) stress, compared with wild type *Pax4* (WT), was examined.
Materials & Methods: The plasmid construct containing either *Pax4* WT or *Pax4* double variants was co-transfected with the plasmid construct containing either insulin or glucagon promoter into βTC3 and α-TC cells. Transcriptional activities were determined. To examine *Pax4* function on cell viability, Pax4 WT, Pax4 double variants or pcDNA3.1/HisB plasmids was transfected into INS-1 cells cultured in normal or high glucose medium. Cell viability from each culture condition was measured by MTT assay.
Results: Transcriptional repressor activities of Pax4 double variants on both promoters were decreased when they were compared with those of Pax4 WT. Pax4 double variants also reduced cell viability in high glucose milieu. However, this finding was not demonstrated in culture medium with normal glucose.
Conclusion: Pax4 WT can protect pancreatic β-cells cultured in high glucose via antioxidant effect or reduction of ER stress while Pax4 double variants impaired transcriptional repressor activities of target genes and reduced β-cell viability in hyperglycemic condition. Thus, the defect of Pax4 double variants may be associated with the deregulation of its transcriptional activities and the reduction of β-cell mass leading to the development of diabetes.

Keywords: *Pax4*, pancreatic β-cells, high glucose, diabetes

Suppression of GANP Causes DNA Damage and Cell Death in p53-insufficient Cholangiocarcinoma Cells

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Introduction: Cholangiocarcinoma (CCA) is a typical inflammation-associated tumor that often accompanies with DNA damages of critical molecules such as p53. Recent studies demonstrated that the inflammation to cholangiocytes causes the high-level expression of an activation-induced cytidine-deaminase (AID) and its binding protein germinal center-associated nuclear protein (GANP).
Objective: To study the effect of *ganp*-knockdown on malignant CCAs and details of molecular mechanism in the induction of apoptosis.
Materials & Methods: CCA cell lines (KKU100, M156, M213 and M214), MMNK-1 cholangiocyte and HeLa were used for si*Ganp* treatment. Cell cycle and DNA damage were determined by BrdU- and alkaline comet-assays. Cell death was quantified by trypan blue staining, AnnexinV/PI staining, and caspase activation. Tumor growth was measured using Balb/c-Rag-2/Jak3 double-deficient mice.
Results: si*Ganp* significantly induced cell death in M156, M213, M214 and HeLa which are p53 insufficient cells but not in MMNK-1 and -KKU100. si*Ganp* decreased the S-phase with G2/M arrest by inducing DNA damages and induced both intrinsic- and extrinsic-pathways in p53-insufficient tumors. z-VAD-fmk could not completely block the si*Ganp*-induced cell death indicating the interaction of both caspase-dependent and -independent mechanisms. si*Ganp* showed apoptotic and necrotic cell death and efficiently prevented the tumor growth *in vivo*.
Conclusion: s*iGanp* treatment induces the strong cell death reaction causing DNA damage and activates caspase-dependent and -independent apoptosis pathways, resulting in cell apoptosis and necrosis. Thus, si*Ganp* is an efficient and selective treatment of p53-insufficient CCA patients.

Keywords: apoptosis, cell cycle, DNA damage, siRNA

Compartmentalized Molecular Machinery in Regulatory T Cell Function

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Introduction: One of the crucial functions of immune system lies in the fine balance between self-tolerance and self-reactivity, failure of this regulation results in myriad of autoimmune diseases and defective tumor immunity. While there are many mechanisms that promote self-tolerance, one of the most important includes $CD4^+CD25^+$ regulatory T cells (Treg). One of the primary Treg targets for suppression is $CD4^+CD25^-$ effector T cells (Teff).
Objective: Antigen presenting cells (APC) are the crucial and main instigators of immune response for both Treg and Teff, however cell biological principles that differentiate Treg and Teff to recognize antigen with the same receptor, but have opposing functions remain unclear.
Materials & Methods: We utilize in-vitro suppression assays and high resolution imaging of human Treg and Teff to visualize subcellular compartmentalization of signaling molecules and its consequences on Treg function.
Results: One aspect of this cell-intrinsic signal transduction diversity is reflected in the unique subcellular distribution of key pro-inflammatory signaling molecules we recently observed in Tregs. This unique localization of molecules is specifically exhibited by human Tregs in diverse contexts, is sensitive to both extracellular signals and subcellular mechanical elements and is tightly correlated with Treg function.
Conclusion: Results obtained from this study will not only offer novel paradigm for understanding signaling compartmentalization in T cells, will also provide insights into Treg trafficking, function and can perhaps be furthered to achieve improvised therapeutic targeting.

Keywords: regulatory T cells, effector T cells, immune tolerance

Blockade of *in vitro* Osteoclastogenesis by the Antimicrobial Peptide LL-37 through Reducing Calcineurin Activity and Inhibiting Nuclear Translocation of Nuclear Factor of Activated T-cells 2

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Introduction: Uncoupled bone resorption leads to net alveolar bone loss in periodontitis. The deficiency of LL-37, the only human antimicrobial peptide in the cathelicidin family, in patients with aggressive periodontitis suggests that LL-37 may play a role in inhibition of osteoclastogenesis and alveolar bone destruction in periodontitis.
Objective: We, therefore, aimed to investigate a novel inhibitory effect of LL-37 on *in vitro* osteoclastogenesis.
Materials & Methods: Human osteoclast progenitor cells were isolated from a buffy coat of blood samples. The cells were cultured in the presence or absence of various concentrations of LL-37 during an *in vitro* induction of osteoclastogenesis.
Results: Non-toxic doses of LL-37 could block multinuclear formation of the progenitor cells and significantly diminish the number of tartrate-resistant acid phosphatase-positive cells and the formation of resorption pits ($p<0.05$), whereas these concentrations induced cellular proliferation, as demonstrated by increased expression of macrophage-colony stimulating factor and of proliferating cell nuclear antigen. Expression of several osteoclast-specific genes, including calcitonin receptor, cathepsin K, matrix metalloproteinase-9, receptor activator of nuclear factor kappaB, and nuclear factor of activated T-cells 2 (NFAT2), a master transcription factor for osteoclast formation, was significantly down-regulated by LL-37 treatment ($p<0.05$). By immunofluorescence and immunoblotting studies, it was shown that LL-37 blocked nuclear translocation of NFAT2, consistent with a significant reduction in the calcineurin activity by LL-37 ($p<0.005$).
Conclusion: Our findings demonstrate that LL-37 inhibits the *in vitro* osteoclastogenesis by decreasing the calcineurin activity, thus preventing nuclear translocation of NFAT2. This study was supported by the TRF (RMU5380014) and the NSTDA (P-10-11290).

Keywords: aggressive periodontitis, cathelicidin, innate immunity, nuclear factor of activated T-cells 2, osteoimmunity

Novel Polymer-bacteriophage Hybrid Complex as a Promising Tool Towards Efficient and Targeted Gene Delivery to Cancer

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Introduction: Previously, we reported the efficacy of an improved version of non-human pathogenic viral vectors for targeted cancer gene therapy. Specifically, the bacteriophage was genetically engineered to display a specific tumour-targeting RGD4C ligand and to carry a CMV promoter-driven transgene cassette flanked by the Inverted Terminal Repeats (ITR) from adeno-associated virus (AAV2). The use of this vector showed significant efficacy in several animal models of cancer as well as in pet dogs with spontaneous tumours. However, inherent limitations in bacteriophage mean that ways of improving it are of considerable interest.
Objective: To generate, characterize and evaluate a hybrid polymer-bacteriophage vector as a novel system for targeted gene delivery to cancer.
Materials & Methods: We formed self-assembled complexes using the established bacteriophage vector and cationic polymers: diethylaminoethyl-dextran (DEAE.DEX.) or poly-D-lysine (PDL), and used them to transduce cancer cells.
Results: We demonstrated that the positively charged complexes adsorb negative charge cell membrane surfaces after which vectors are endocytosed into the cells via integrin receptors overexpressed on cancer cell membranes. A study of the expression of green-fluorescent and luciferase reporter genes has confirmed that such hybrid vectors display markedly high transduction efficiencies in several cancer cell lines. Application of the targeted hybrid complex carrying the cytotoxic gene '*HSV*tk' resulted in brain glioma cell eradication in combination with ganciclovir treatment.
Conclusion: This polymer-bacteriophage complex showed superior gene delivery over the established vector and can thus be regarded as a new generation of hybrid vector systems that has promise in cancer gene therapy.

Keywords: bacteriophage, gene therapy, cancer, polymer

A-18

The Inhibitory Activity of RNA Elements in the Late 3' Untranslated Region of Human Papillomaviruses and Bovine Papillomaviruses

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Introduction: Infection by papillomaviruses (PVs) commonly causes wart, which in some rare cases may develop to malignancy. It is known that viral late gene expression is controlled post-transcriptionally in response to epithelial differentiation. The regulation of gene expression is mediated by the interaction between RNA processing factors and regulatory elements locating both within the coding sequences and in the late 3' untranslated region (UTR) of virus genome.
Objective: The aim of this study was to determine the inhibitory activity of the late 3'UTR of six different PVs.
Materials & Methods: The 3'UTR sequences of six PVs which composed of high-risk HPVs, HPV-16 and -31, low risk HPVs, HPV-6 and -11, and BPVs, BPV-1 and 2 were used in the study. The nucleotides, covering the 3' end of L1 ORF to late poly (A) signal, were analyzed the phylogram using clustalW2. Inhibitory activity of each 3'UTR PV on gene expression was examined using RT-PCR with a beta-galactosidase reporter system.
Results: As expected, the resulting phylogenetic tree classified the investigated viruses into three distinct groups, composing of high risk, low risk and bovine-infected virus types. Results from RT-PCR indicated that all late UTRs can inhibit beta-galactosidase expression, but to different degrees.
Conclusion: The high risk types, HPV-16 and -31 displayed the highest inhibitory activity while the lowest and second lowest inhibitory activities were observed from the low risk types, HPV-11 and BPV-4 late UTRs. It is likely that inhibitory efficiency of each virus type is consistent with the groups classified in the constructed phylogram.

Keywords: papillomaviruses, L1 gene, 3' untranslated region

Neuroprotective Effect of 4,5-dianilinophthalimide Against Serum Deprivation-induced Neuronal Injury in PC12 Cell Line

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Introduction: Neuronal cell death by apoptosis is the end point of many neurological disorders, including Alzheimer's, Parkinson's, and stroke/trauma. The serum deprivation-induced cell death in cultured PC12 cells represents a useful *in vitro* model for the study of brain ischemia and neurodegenerative disorders.
Objective: In this study, we aimed to investigate neuroprotective effects of 4,5-dianilinophthalimide (DAPH) on serum deprived PC12 6/15 cell line.
Materials & Methods: The neuroprotective activity was evaluated using dopaminergic neuron model PC12 6/15 cell line. Neuronal differentiation of the cells were induced with 100 ng/mL NGF for 1 day. Serum-deprived differentiated cells were treated with DAPH (1, 5, 10, 20, 40 and 80 μmol/L). Cell viability was determined by MTT assay. Apoptosis antibody array was performed to identify changes in apoptosis related proteins. Also, expression of stress markers such as p-HSP27 and p-SAPK/JNK were checked by Western blot.
Results: The MTT assay demonstrated that DAPH has growth inhibitory effect in a concentration-dependent manner (LD50: 20 μmol/L at 24h). Administration of DAPH at nontoxic dose (10 μmol/L, viability 85%) resulted in partially increased expression of several anti-apoptotic proteins such as Blc-2, Blc-w, survivin, livin and c-IAP2 (P>0,05). In addition to this, Western blot analysis revealed that expression of stress markers p-HSP27 and p-SAPK/JNK were decreased after DAPH treatment (P<0,05 and P>0,05, respectively).
Conclusion: The experimental results suggest that nontoxic dose of DAPH protects PC12 cells against serum-deprivation induced stress and apoptosis. Our findings might raise the possibility of potential therapeutic application of DAPH for several neurodegenerative disorders.

Keywords: DAPH, serum deprivation-induced apoptosis, PC12 cell line

Poster Presentation Award A-20

A New 2-pyrone Derivative, 5-bromo-3-(3-hydroxyprop-1-ynyl) -2H-pyran-2-one, a 2-pyrone Derivative, Suppresses Oncogenic K-Ras-induced Malignant Progression in Breast Epithelial Cells

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Introduction: K-Ras acts as molecular switch to transduce extracellular signals to the nucleus. Aberrant K-Ras activation is often associated with cancer progression. 2-Pyrones, six-membered cyclic unsaturated esters that are highly abundant in bacteria, plant and animal systems have shown to have anticancer activity in human leukemic cells. However, the potential of these compounds to modulate the malignant progression in human epithelial cells remain unknown.
Objective: We aimed to examine the effect of a new 2-pyrone derivative, 5-bromo-3-(3-hydroxyprop-1-ynyl)-2H-pyran-2-one (BHP) on K-Ras induced-malignant progression in human epithelial cells.
Materials & Methods: Mutant G13D K-Ras was transduced to breast epithelial MCF10A cells. Migration or invasion assays were done in Trans-well that was pre-coated with matrigel or not. 10 μM BHP was treated for 2-days and signaling pathways were analyzed by immunostaining. Cell death was measured by FACS analysis after staining with propidium iodide.
Results: K-Ras-expressing cells displayed more migratory and invasive phenotypes, concomitant with epithelial mesenchymal transition (EMT) and resistance to anticancer treatment, compared to parental cells. Notably, treatment with BHP inhibited the K-ras-induced malignant transformation. BHP treatment caused to decrease EMT-transcription factors Zeb1, Snail and Slug along with a decrease of mesenchymal markers N-cadherin, Vimentin in K-Ras-transformed cells. Also, BHP treatment greatly enhanced the sensitivity of K-ras-transformed cells to anticancer treatments. Importantly, BHP treatment inhibited PI3K/AKT and Ras/Raf-1/ERK signaling pathways, thereby suppressing K-Ras-induced malignancy.
Conclusion: This study suggests that BHP suppresses malignant progression induced by oncogenic K-Ras in breast epithelial cells through inhibition of PI3K/AKT and Ras/Raf-1/ERK signaling pathways.

Keywords: K-Ras, 2-pyrone derivative, EMT

Ca^{2+}-related ER-associated Molecules Regulated by Pro-inflammatory Molecules

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Introduction: Pro-inflammatory molecules secreted from macrophages regulate tumor growth.
Objective: In this study, we investigated that pro-inflammatory molecules could regulate endoplasmic reticulum (ER)-related cell death signal in tumor cells.
Materials & Methods: Human brain tumor cells were treated with a variety of pro-inflammatory molecules including TNF-α, H_2O_2, Nitric Oxide, LPS or thapsigargin.
Results: Among them, Nitric Oxide, H_2O_2, or thapsigargin treatment resulted in the decrease of cell viability as indicated by terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling and the increased cytosolic Ca^{2+}. In contrast, LPS and TNF-α did not affect both cell viability and cytosolic Ca^{2+}. Nitric oxide, H_2O_2, and thapsigargin increased ER-resident protein IRE1-α and Ca^{2+}-sensitive p-CREB expressions. The elevation of Ca^{2+} was necessary for activation of IRE1-α. IRE1-α induction coincided with those of p-JNK1/2. The level of p-ERK1/2 as well as p-JNK1/2 also increased. On the other hand, TNF- and LPS which did not affect cell viability did not increase IRE1- and p-JNK1/2. It suggests that IRE1- and p-JNK-1/2 increased by Ca^{2+} disturbance depleting Ca^{2+} from the ER is related to ER-mediated cell death.
Conclusion: Experimental evidences indicate that the elevation of calcium is necessary for pro-inflammatory molecules-mediated cell death through IRE1-related pathway.

Keywords: proinflammatory molecules, Ca^{2+}, ER

Parkin Induces Expression of MMP-3 in Human Cervical Cancer Cells

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Introduction: Parkin is known to be a tumor suppressor protein. Previously, we determined that parkin expression restores susceptibility to TNF-alpha-induced death of HeLa cells, a human cervical cancer cell line resistant to TNF-alpha-induced cell death. MMP-3 is a zinc-dependent protease recently reported to activate intracellular apoptotic signaling. In this study we examined the regulation of MMP-3 expression by parkin in TNF-alpha-treated HeLa cells. Furthermore, we investigated the signaling pathway(s) involved in parkin-induced expression of MMP-3.
Objective: To determine the role of parkin on expression of MMP-3 in HeLa cells. To identify cell signaling pathway(s) involved in parkin-induced MMP-3 expression.
Materials & Methods: Parkin gene was introduced via recombinant adenoviral vector and TNF-alpha was administered 24 hours post-parkin-introduction. MMP-3 mRNA level was examined by RT-PCR analysis. Chemical inhibitors were used to identify cell signaling pathway(s).
Results: We found that MMP-3 expression was induced by TNF-alpha but it was decreased in TNF-alpha resistant HeLa cells. However, in HeLa cells expressing parkin MMP-3 expression remained elevated. Furthermore, MMP-3 expression was up-regulated in a parkin dose-dependent manner. Using chemical inhibitors of cell signaling pathways, we found that the MEK-1 (PD98059), PI3K (LY294002), p38 MAPK (SB203580), and JNK inhibitors alleviated parkin-induced up-regulation of MMP-3.
Conclusion: In TNF-alpha resistant human cervical cancer HeLa cells, parkin expression induced prolonged expression of MMP-3 via MEK-1, PI3K, MAPK, and JNK pathway. We speculate that MMP-3 expression is implicated in parkin-induced cell death in TNF-alpha treated HeLa cells.

Keywords: parkin, TNF-alpha, MMP-3, cervical cancer, tumor suppressor

Involvement of Decoy Receptor 3 in Epidermal Keratinocyte Commitment to Terminal Differentiation

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Introduction: Decoy receptor 3 (DcR3/TNFRSF6B), a member of tumor necrosis factor receptor superfamily, is a soluble receptor of Fas ligand (FasL/TNFSF6/CD95L), LIGHT (TNFSF14) and TNF-like molecule 1A (TL1A/TNFSF15). The pleiotropic functions of DcR3 in inflammatory disorders, autoimmune diseases and carcinogenesis have been widely investigated. However, the functional roles of DcR3 in cutaneous biology are still limited.
Objective: To investigate the regulation of DcR3 expression in epidermal keratinocytes and further explore the biologic functions of DcR3 in growth and differentiation of epidermal keratinocytes.
Materials & Methods: Primarily cultured epidermal keratinocytes isolated from human skin were used for studies. Western blotting, quantitative real-time PCR and ELISA were used to evaluate the regulation of DcR3 expression in epidermal keratinocytes. By knocking down DcR3 mRNA, we further explored the biologic roles of DcR3 in epidermal keratinocytes
Results: DcR3 was constitutively expressed in non-confluent and proliferating primary human epidermal keratinocytes but was progressively suppressed during the process of terminal differentiation by different inducers. However, inflammatory cytokines could promote the DcR3 expression in keratinocytes. When knocking down DcR3 expression, the induction of terminal differentiation markers including keratin 10, loricrin and profilaggrin were enhanced but the expression of involucrin and transglutaminase 1 were inhibited.
Conclusion: DcR3 expression in keratinocytes is regulated during the differentiation process and in inflammatory conditions. The expression level of DcR3 in keratinocytes can modulate the pattern of terminal differentiation, which may imply its roles in skin disorders.

Keywords: keratinocytes, decoy receptor 3, differentiation

Common Polymorphisms in Inflammatory Genes Interleukin (IL)-6, IL-8, Tumor Necrosis Factor-alpha (TNF-α), Intercellular Adhesion Molecule-1 (ICAM-1) and Peroxisome Proliferators-activated Receptor-gamma (PPAR-γ) and Their Risk Association in Malaysian Sporadic Colorectal Cancer Patients

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Introduction: Sporadic Colorectal cancer (CRC) results form complex interaction between environmental and genetic predisposition factors. Chronic inflammation has been recognized as a major risk factor for cancers including CRC. If inflammation constitutes one of the molecular networks underlying susceptibility to CRC, genes which mediate inflammatory response might be a group of candidate genes for CRC predisposition. We hypothesized that single nucleotide polymorphisms (SNPs) of inflammatory respsonse genes could be logical candidates as genetic determinants of CRC risk.
Objective: This case-control study aimed to investigate the genotype frequencies of 5 inflammation response gene SNPs such as *IL-6* G174C, *IL-8* T251A, *TNF-α* G308A, *ICAM-1* K469E, and *PPAR-γ* C34G in 255 Malaysian sporadic CRC patients and 255 normal controls and to evaluate their influential role in modulating CRC risk.
Materials & Methods: Genomic DNA extracted from 510 study subjects were genotyped employing PCR-RFLP and allele-specific PCR techniques, and risk association was assessed computing Odds Ratio (ORs) with corresponding 95% CI.
Results: Among the 5 SNPs studied, the variant genotypes of *IL-8* and *TNF-α* were found to be overrepresented among CRC patients and consequently showed significantly higher risk association for CRC when analyzed singly (OR: 3.600, CI: 1.550-8.481, *P*=0.001 and OR: 3.921, CI: 1.518-10.526, *P*=0.001 respectively) and also in few combinations with other SNPs.
Conclusion: SNPs in inflammation response genes *TNF-α* and IL-8 contribute significantly to CRC risk and could be considered as potential CRC genetic predispotion factors. Results also support inflammation mediated colorectal carcinogenesis pathway.

Keywords: inflammation response genes, SNPs, colorectal cancer risk

Factor Coagulation 5 – the Determinant in Nasal Polyps Response to Endoscopic Simple Polypectomy and Intranasal Glucocorticoid: A Pathway for Resolution of the Chronic Inflammation

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Introduction: Nasal Polyps (NP) is the ultimate sinonasal inflammation predisposed by multiple risk factors. We studied mRNA gene expression profiles in NP of pre- and post-protocol treatment by endoscopic simple polypectomy and followed by a 6-week intranasal glucocorticoid.
Objective: To obtain the evidence of designated protocol treatment of NP and to understand the resolution of inflammation process.
Materials & Methods: Twenty-nine patients with naïve bilateral NP were included into the study. Subjects classified by clinical criteria into responder group in 16 subjects and 13 non-responder subjects.
Results: Sixty-two functional genes were identified, where 44 genes were up-regulated at least 2-folds, and 18 genes were down-regulated. Six of the most over-expressed genes were selected and retested by means of real-time RT-PCR. The responder group showed significantly higher transcriptional activity of STATH (statherin), PIP (prolactin-induced protein), LTF (lactoferrin) and F5 (factor coagulation 5). In the non-responder group, significantly higher expression of DMBT-1 (deleted malignant brain tumor-1) and HP (haptoglobin) genes were detected. Multivariate analysis using stepwise-forward (likelihood ratio) method of logistic regression to assess the determinant among independent variables of age, gender, NP grade, bacterial existence, MMP-9 and F5 genes, showed that F5 gene was found as significant determinant in response to protocol treatment.
Conclusion: F5 was plausibly a response to injury caused by endoscopic simple polypectomy intervention by its initial purpose to have a wider contact mucosa in increasing absorption of intranasal glucocorticoid. The inducible F5 strengthen endogenous anti-inflammatory mediators activity in resolution pathway to stop ongoing persistence inflammation of NP.

Keywords: gene expression, inflammation network, nasal polyps

A-26

An Integrative Genomic Analysis Identifies a Subset of Colorectal Cancers with Oncogenic Dependence on Tri-snRNP Spliceosome Activity

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Introduction: Colorectal cancer is a leading cause of cancer related death worldwide. Genetic alterations that drive colorectal cancer progression such as KRAS, APC loss or MYC are not easily druggable suggesting that further effort is required to identify therapeutic targets for colorectal patients.
Objective: In this study, we used structural and functional genomic analyses to identify genes that are both amplified and necessary for colon cancer growth.
Materials & Methods: A high throughput RNAi loss of function screen was conducted on ~1300 genes and miRNAs that were overexpressed and amplified in colon cancer.
Results: Using integrative genomic techniques, we identified PRPF6, a tri-snRNP spliceosome component, as being amplified, overexpressed and necessary for proliferation and tumor growth in a subset of colon cancer cell lines. We show that colon cancers that overexpress PRPF6 concomitantly upregulate other tri-snRNP spliceosomal components and become dependent on the tri-snRNP complex for proliferation. Using next generation RNA-seq analysis and splicing reporters, we show that loss of tri-snRNP activity impairs splicing in a subset of genes involved in cell growth rather than global changes to the transcriptome. Remarkably, loss of function mutations in several tri-snRNP components have been identified in a tissue-specific familial syndrome called Retinitis Pigmentosa.
Conclusion: We hypothesize, then, that therapeutic inhibition of the tri-snRNP complex may be a tractable approach for cancers, that similar to the retina, specifically depend on tri-snRNP function for gene-specific splicing.

Keywords: integrative genomics, colon cancer, spliceosome

HAMLET and BAMLET Induces Cell Death of Primary Effusion Lymphoma

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Introduction: Primary Effusion Lymphoma (PEL) is a subtype of non-Hodgkin's B-cell lymphoma which mainly presents in patients with advanced AIDS. PEL cells grow as a lymphomatous effusion in body cavities and are infected with Kaposi sarcoma-associated herpes virus (KSHV) and patients are resistant to chemotherapy. HAMLET (human -lactalbumin made lethal to tumor cells) and BAMLET (bovine -lactalbumin made lethal to tumor cells), a complex of partially unfolded -lactalbumin and oleic acid, kills a wide range of tumor cells.
Objective: We report finding of anti-tumor effect of HAMLET and BAMLET on PEL cell lines *in vitro* and *in vivo*.
Materials & Methods: A methylthiotetrazole (MTT) assay was performed to determine anti-proliferation effect of BAMLET and HAMLET on PEL cell lines (BCBL-1, BC-1, BC-3 and TY-1). Induction of apoptosis was detected by Annexin V assay. Western blot analysis and flow cytometry analysis was performed to reveal mechanism of cell death. *In vivo* study, we inoculated BCBL-1 intraperitonially into NOD/Scid/jak3 deficient mice, and treated these mice with BAMLET or PBS three times a week.
Results: BAMLET and HAMLET treatment significantly suppressed proliferation of PEL cell lines and induced apoptosis via caspase dependent manner. BAMLET also induced reactive oxygen stress, autophagy and JNK activation. *In vivo* study, intraperitoneal administration of BAMLET reduced the amount of ascites and inhibited splenomegaly without significant systemic toxicity.
Conclusion: These results suggest that HAMLET and BAMLET could be an effective and attractive reagent for PEL treatment.

Keywords: primary effusion lymphoma, BAMLET and HAMLET, autophagy, animal model

A-28

Heparin Antagonist Virus-like Nanoparticles

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Introduction: Heparin has myriad functions *in vivo* including angiogenesis, metastasis, and cell-cell communication. The structure consists of repeating disaccharide units with discrete regions of sulfation, thus representing a multivalent polymer. Antagonists that can modulate heparin activity can be of enormous clinical use.
Objective: Using the polyvalent virus-like platform afforded by bacteriophage Qβ, we sought to construct heparin antagonists by generating cationic particles via mutation.
Materials & Methods: Guided by the crystal structure, the Qβ coat protein was mutated at selected positions on the capsid surface using standard PCR techniques. Expression and isolation of intact capsids was accomplished using *E. coli*. Particle integrity was confirmed by MALDI-MS, FPLC, and SDS-PAGE. For preliminary assays of anti-heparin activity we utilized the APTT clotting assay.
Results: A series of particles were generated with increased surface charge via substitution of residues for arginine at various position on the capsid surface. Of these we found one, T18R, that could be generated readily and was effective at consistently reversing heparin anticoagulant activity. In comparison to protamine, the only clinically approved antidote for reversal of heparin-induced bleeding, T18R displayed similar efficacy while showing none of protamine's acutely toxic side effects *in vitro*.
Conclusion: The anti-heparin activity displayed by T18R opens the door for further work that probes the specificity of the interaction. Future work will look at biophysical properties of the interaction, and generating new particles that will help elucidate rules for the heparin-virus interaction.

Keywords: heparin, virus-like particle, coagulation

Regulation of MCP-1 and CCR2 Expression in Triglyceride Treated PMA-derived THP-1 Macrophages

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Introduction: Triglycerides (TG) are implicated in the development of atherosclerosis. A key contributing factor for atherosclerosis is the migration of macrophages to atherosclerotic lesions, subsequent formation of foam cells and macrophage cell death. MCP-1 is a major chemoattractant for macrophages to atherosclerotic lesions. In this report, we examined the expression profile of MCP-1 and the cognate receptor, CCR2, in PMA-derived THP-1 human macrophages in response to TG treatment. We further investigated the signaling pathway(s) involved in regulation of MCP-1 and CCR2 expression.
Objective: To determine the role of TG on expression of MCP-1 and CCR2 in THP-1 macrophages. To identify cell signaling pathways involved in regulation of MCP-1 and CCR2 expression.
Materials & Methods: PMA-derived THP-1 human macrophages were stimulated with TG and the expression of MCP-1 and CCR2 examined by RT-PCR analysis. Chemical inhibitors were used to identify cell signaling pathway(s) involved in regulation of MCP-1 and CCR2 expression.
Results: We found that treatment of THP-1 macrophages with TG down-regulated MCP-1 expression in a time and dose-dependent manner. PMA treatment alone did not affect MCP-1 expression. Using chemical inhibitors of cell signaling pathways, we found that the NF-B inhibitor (Bay 11-7085) inhibited downregulation of MCP-1. CCR2 expression decreased after TG treatment in THP-1 macrophages and the PKC inhibitor (RO-31-7549) alleviated TG-induced down-regulation of CCR2.
Conclusion: In human THP-1 macrophages, TG treatment resulted in down-regulation of MCP-1 expression via activation of the NF-κB pathway whereas down-regulation of CCR2 expression occurred via activation of the PKC pathway.

Keywords: atherosclerosis, triglyceride, MCP-1, CCR2, macrophages

Role for DYRK2 in Cell Cycle Control and Tumor Progression

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Introduction: Dysregulation of the G1/S transition in the cell cycle contributes to tumor development. The oncogenic transcription factors c-Jun and c-Myc are indispensable regulators at this transition, and their aberrant expression is associated with many malignancies. Degradation of c-Jun/c-Myc is a critical process for the G1/S transition, which is initiated upon phosphorylation by glycogen synthase kinase 3 β (GSK3β). However, a specific kinase or kinases responsible for priming phosphorylation events that precede this GSK3β modification has not been definitively identified.
Objective: To identify a specific kinase responsible for priming phosphorylation events that precedes GSK3β modification.
Materials & Methods and Results: We found that the dual-specificity tyrosine phosphorylation-regulated kinase DYRK2 functions as a priming kinase of c-Jun and c-Myc. Knockdown of DYRK2 in human cancer cells shortened the G1 phase and accelerated cell proliferation due to escape of c-Jun and c-Myc from ubiquitination-mediated degradation. In concert with these results, silencing DYRK2 increased cell proliferation in human cancer cells, and this promotion was completely impeded by co-deprivation of c-Jun or c-Myc *in vivo*. We also found marked attenuation of DYRK2 expression in multiple human tumor samples. Downregulation of DYRK2 correlated with high levels of unphosphorylated c-Jun and c-Myc and, importantly, with invasiveness of human breast cancers.
Conclusion: The results reveal that DYRK2 regulates tumor progression through modulation of c-Jun and c-Myc.

Keywords: DYRK2, c-Jun, c-Myc, GSK3β, ubiquitination, breast cancer

Therapeutic Potential of DMHCA for Pulmonary Arterial Hypertension

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Introduction: Severe pulmonary arterial hypertension (PAH) is a fatal, multifactorial vascular disease that is characterized by lung vascular remodeling, high pulmonary blood pressure and right ventricular hypertrophy. Currently there are no effective therapies and new therapeutical strategies are urgently needed.
Objective: PAH is known to be associated with immune dysfunction and inflammation. Recently we found that in PAH function of Liver X receptor (LXR) (a known regulator of lipid metabolism and inflammation in macrophages) is impaired. Here we investigate whether treatment with synthetic steroidal LXR activator N,N-dimethyl-3b-hydroxy-cholenamide (DMHCA) encapsulated into microparticles can attenuate inflammation and the development PAH.
Materials & Methods: Sprague Dawley (SD) rats were exposed to hypobaric hypoxia (18000 ft = 5,000 m latitude) for 3 weeks. Rats were divided into 3 groups: 1) untreated (Hx); 2) VEGF receptor inhibitor SUGEN5416 (20mg/kg) treated; 3) one week prior the exposure to SUGEN5416 and hypoxia, treated with DMHCA-microparticles (DMHCA-P); 4) DMHCA-P alone. Pulmonary artery pressures (PAP), hematocrit, right ventricle hypertrophy, inflammation and LXR expression levels were determined.
Results: Exposure to hypoxia and SU5416/hypoxia significantly increased hematocrit values and RV/(LV+S) ratios when compared to normoxic control group and changed the LXR receptor (α and β) ratio. Pretreatment with DMHCA blocked the development of PAH, normalized the hematocrit, reversed LXR expression ratio in macrophages and significantly decreased inflammation.
Conclusion: Our findings suggest that LXR is a potential therapeutic target for PAH. LXR activation might have a fundamental protective effect against the development of PAH. Funded by AHA 0735388N, 11GRNT7520020, and Emphysema Research Fund

Keywords: inflammation, macrophages, vascular lesions, pulmonary hypertension, LXR

Human Macrophage and Dendritic Cell-specific Silencing of HMGB1 Ameliorates Sepsis in a Humanized Mouse Model

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Introduction: During sepsis, HMGB1 secreted by macrophages and dendritic cells induces a cytokine storm that lead to multiple organ failure and mortality.
Objective: Targeted delivery of siRNA to suppress HMGB1 in human macrophages and dendritic cells to suppress sepsis in humanized mice.
Materials & Methods: We induced sepsis by performing ceacal ligation and puncture (CLP) in WT mice, highly immunodeficient NOD/SCID/IL2Rγ-/- mice and NOD/SCID/IL2Rγ-/- mice transplanted with human hematopoietic stem cells (humanized mice). Following CLP, humanized mice were treated with control or HMGB1 siRNA using a short AchR binding peptide (RVG-9R) peptide to deliver siRNA to human macrophages and dendritic cells.
Results: Following CLP, as compared to immunocompetent WT mice, NOD/SCID/IL2Rγ-/- mice did not show high levels of serum HMGB1 or murine proinflammatory cytokines and were relatively resistant to sepsis-induced mortality. In contrast, NOD/SCID/IL2Rγ-/- mice transplanted with human hematopoietic stem cells (humanized BLT mice) showed high serum levels of HMGB1 as well as multiple human, but not murine proinflammatory cytokines and uniformly succumbed, suggesting human cytokines are sufficient to induce organ failure in this model. Targeted delivery of HMGB1 siRNA to human macrophages and dendritic cells effectively suppressed secretion of HMGB1, reduced the human cytokine storm and rescued humanized mice from CLP-induced mortality.
Conclusion: HMGB1 siRNA might provide a treatment strategy for human sepsis and RVG-9R provides a tool to deliver siRNA to human macrophages and dendritic cells that could potentially be used to suppress a variety of human inflammatory diseases.

Keywords: inflammation, cytokine storm, sepsis, siRNA treatment, HMGB1

Alterations of Toll-like Receptor Signaling Pathway in Gliomas

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Introduction: All cells of the nervous system express an abundance of innate immune receptors, among them toll like receptors (TLR) are central to neuroinflammation. Currently little is known about differences between proper and improper neuroinflammation and their role in cancer.
Objective: Our aim was to analyze the alterations in TLR signaling pathway among the subtypes of gliomas. We have performed a systematic analysis of the intrinsic organization of complex glioma transcriptome (Cancer Res. PMID: 21159630) and now analyze position of TLR signaling pathway genes among others (RFFR grant 10-04-01385a).
Materials & Methods: Data on whole genome expression used in the present study are available from GEO database under ID GSE16011 (obtained with AffymetrixU133 Plus 2.0), TLR pathway participants list was combined from sources: KEGG, Pathway Central database, recent review (PMID: 22721918). Differentially expressed genes (DEGs) were detected with application MeV (TM4) using t-test with Bonferroni correction. Analysis of gene expression correlations was made in R (CRAN).
Results: 1) Expression of TLR signaling components increases in gliomas, comparing to normal brain tissues. The classes with worse prognosis are characterized with higher number of genes with altered expression. 2) Most of TLRs display increased expression in cancer samples. 3) TLR expression alterations correlate with alterations of specific markers of immune and glial cells, providing the hints on cellular mechanisms of inflammation in gliomas. 4) Mechanisms of TLR signaling alterations in gliomas tend to be subtype specific.
Conclusion: TLR signaling alteration involves different mechanisms in gliomas of different types and presumably contributes to poor prognosis of the condition.

Keywords: innate immunity, inflammation, toll-like receptors, glioma, transcriptome

Cancer Stem Cells: Novel Chemopreventive Targets for Pancreatic Cancer

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Introduction: Pancreatic cancer is the fourth leading cause of mortality in the United States and no significant treatment is currently available. The existence of a small population of cancer stem cells (CSCs) is responsible for tumor initiation, metastasis and resistance to chemotherapy and radiation.
Objective: The objective of this work is to understand the mechanisms by which purified crocetinic acid, a carotenoid molecule isolated from saffron, inhibits tumorigenesis of pancreatic cancer.
Materials & Methods: Pancreatic CSCs can be allowed to divide and grow in ultra-low binding tissue culture dishes to form multicellular spheroids called pancospheres and treated with or without crocetinic acid in pancreatic cancer models.
Results: Treatment with purified crocetinic acid decreased the number and size of the primary and secondary pancospheres in a dose dependent manner. Aberrant activation of Sonic Hedgehog (Shh) signaling pathway has been associated with renewal of cancer stem cells, and in the development of several solid cancers. Shh upon binding to its receptor patched, allows smoothened to accumulate and activate Gli transcription factor. Crocetinic acid inhibited the expression of both Shh and smoothened in CSCs with concomitant reduction of the expression of a novel pancreatic CSC marker, DCLK-1 (Doublecortin Calcium/Calmodulin-Dependent Kinase-1). Furthermore, it inhibited the expression of patched-1 and Gli-1, downstream targets of the hedgehog signaling pathway. Crocetinic acid also inhibited tumor formation in pancreatic cancer *in vivo* xenograft models.
Conclusion: Taken together, crocetinic acid effectively inhibits pancreatic CSCs by down regulating the sonic hedgehog pathway, thereby inhibiting tumorigenesis.

Keywords: cancer stem cells, chemoprevention, natural products, crocetinic acid, Sonic Hedgehog

Diabetes Aggravates Oxidative Stress and Protein Modification in Tissues of Aged Rats: Results of Treatment with Pyridoindole Antioxidant SMe1EC2

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Introduction: Comprehensive evidence suggests an important role of increased attack of oxidized lipid and sugar intermediates to the proteins in the etiology of aging and diabetes. Antioxidants are promising tools to maintain cellular redox homeostasis, inhibiting excessive formation of advanced glycoxidation end products, advanced lipid peroxidation end products (AGEs/ALEs) and their interactions with proteins.
Objective: We compared tissue levels of AGEs-, 4-HNE-protein adducts and 3-nitrotyrosine (3-NT) by ELISA and the consequences of treatment with a novel pyridoindole antioxidant SMe1EC2 (2-ethoxycarbonyl-8-methoxy-2,3,4,4a,5,9b-hexahydro-1H-pyrido[4,3-b] indolinium dichloride) in young, aged and aged diabetic animals.
Materials & Methods: Diabetes induced by streptozotocin injection in rats with 13-15 months old. SMe1EC2 treatment (10 mg/kg/day) was applied during 4 months to old diabetic rats.
Results: AGE- and HNE-protein adduct levels were increased in brain, ventricle and kidney of aged rats. Addition of diabetes to the aging resulted in a significant increase in oxidized protein adduct levels in lens and liver. SMe1EC2 completely protected against diabetes-induced increase in HNE-protein adducts in lens, and aging+diabetes-induced increase in AGE-protein adducts in kidney ($p<0.001$). SMe1EC2 partly inhibited HNE- and AGE-protein adduct levels in brain and liver of aged diabetic rats ($p<0.05$). 3-NT was increased by aging only in brain. Diabetes aggravated 3-NT increase in kidney, lens and ventricle of old rats, while SMe1EC2 has no protective effect.
Conclusion: Diabetes is a potent substrate for accelerated protein oxidation even in the presence of aging. SMe1EC2 acts as inhibitory factor on AGEs/ALEs formation, mediating its cellular mechanisms on protection of tissue functions. (GÜ project no: 01/2011-09).

Keywords: pyridoindole antioxidant SMe1EC2, protein oxidation, diabetes, aging

Evaluation of Matrix Metalloproteinases-2 and Tissue Inhibitor of Metalloproteinases-2 in Oral Submucous Fibrosis and Their Correlation with Disease Severity

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Introduction: Oral submucous fibrosis, a potentially malignant oral lesion, is a form of pathological fibrosis affecting the oral mucosa. It results from an imbalance in equilibrium of the normal process of synthesis and degradation of extracellular matrix. Matrix metalloproteinases and its inhibitors play important role in remodeling of the extracellular matrix which are important in progression and pathogenesis of potentially malignant lesions to malignancy.
Objective: To evaluate the expression and distribution of Matrix metalloproteinases-2 (MMP-2) and Tissue inhibitor of metalloproteinases-2 (TIMP-2) in different grades of Oral Submucous Fibrosis (OSF).
Materials & Methods: An immuno-histochemical analysis of MMP-2 and TIMP-2 was performed on 30 histopathologically confirmed specimens of OSF (Grade II and III) cases of patients having habit of chewing areca nut.
Results: All Grade III and only 71.5% for MMP-2 and 64.3% for TIMP-2 of Grade II cases showed positivity. Expression of both MMP-2 and TIMP-2 were found to increase with increasing grades of OSF. Between two grades of OSF, statistically significant differences were noted in expression of TIMP-2 in lamina propria, deep connective tissue and supra basal layers ($p<0.05$) and basal and supra basal layers for MMP-2 ($p<0.05$).
Conclusion: The simultaneous increase in expression of MMP-2 and TIMP-2 with advancing grades of OSF can provide a basis for considering the proteases as important mediators in the pathogenesis and progression of OSF which could aid in identifying the aggressiveness of the condition and elucidate its role in its malignant transformation.

Keywords: matrix metalloproteinases, oral submucous fibrosis, immunohistochemical

Expansion and Function of γδ T Cells Stimulated with IL-18: Role of NK Cells

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Introduction: Zoledronic acid has shown benefits when added to adjuvant endocrine therapy for patients with early-stage breast cancer or to standard chemotherapy for patients with multiple myeloma. Although γδ T cells may contribute to this additive effect, the responsiveness of γδ T cells from early-stage breast cancer patients has not been fully investigated.
Objective: In this study, we determined the number, frequency, and responsiveness of Vγ2Vδ2 T cells from early- and late-stage breast cancer patients and examined the effect of IL-18 on their *ex vivo* expansion.
Materials & Methods: Breast cancer patients were enrolled after institutional review board approval and with written informed consent. PBMCs were purified and stimulated with Zol/IL-2 or Zol/IL-2/IL-18 for 2 to 10 days. Expanded cells were assessed on FACS and the production of IFN-γ and TNF-α measured through ELISA.
Results: The responsiveness of Vγ2Vδ2 T cells from patients with low frequencies of Vγ2Vδ2 T cells was significantly diminished. IL-18, however, enhanced *ex vivo* proliferative responses of Vγ2Vδ2 cells and helper NK cells from patients with either low or high frequencies of Vγ2Vδ2 T cells. Cell-to-cell contact between γδ T and helper NK cells appeared to promote expansion of γδ T cells. Exogenous IL-18 markedly enhanced IFN-γ and TNF-α production from PBMC stimulated by Zol/IL-2, whereas the addition of an anti-IL-18Rα mAb reduced cytokine production.
Conclusion: These results demonstrate that Zol elicits immunological responses by γδ T cells from early-stage breast cancer patients and IL-18 enhances proliferative responses and effector functions of γδ T cells in the context of helper NK cells.

Keywords: zoledronic acid, γδ T cells, IL-18

Cancer Multidrug Resistance - Role of Membrane Transporters

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Introduction: Multidrug resistance is a major obstacle in cancer chemotherapy. The key membrane transporters causing this phenomenon are the ABC multidrug transporters, ABCB1, ABCC1 and ABCG2. Human ABCG2 is a plasma membrane glycoprotein that provides protection against xenobiotics and causes drug resistance in cancer. Since up-regulation of the EGF receptor dependent signaling plays an important role in several types of cancer, EGFR inhibition by small molecules is widely applied at the clinics.
Objective: We have studied the potential role of the ABCG2 protein in cancer drug resistance, and the chemotherapy resistance caused by the expression of this multidrug transporter in cancer stem cells. We focused on the potential role of ABCG2 expression on the effects of various EGFR inhibitors and examined clinically relevant inhibitors of the EGFR dependent pathways.
Materials & Methods: We have generated several cancer cell lines which are known to depend on the EGFR activations and overexpressed ABCG2 in these cell types.
Results: We found that the presence of ABCG2 significantly modified the intracellular effects of numerous signaling inhibitors. On the other hand, some of these agents also inhibited ABCG2 function, thereby re-sensitizing multidrug resistant cancer cells to other cytotoxic agents.
Conclusion: We describe a complex interplay between clinically relevant small molecule inhibitors of the EGFR signaling pathway and ABCG2, which may influence drug resistance of cancer cells and especially of cancer stem cells.

Keywords: ABC multidrug transporters, ABCG2 protein, EGF receptor inhibitors

CpG-ODN-induced PAI-1 Expression *via* MEK1/2-ERK- and JNK-dependent and NF-κB-independent Signals

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Introduction: TLR-9 recognizes CpG-oligodeoxynucleotide (CpG-ODN), which mimics bacterial DNA containing non-methylated CpG-motifs, and activates macrophages in innate immune response. Plasminogen activator Inhibitor-1 (PAI-1) deficient mice show reduced macrophages migration during inflammation. However, the regulation of PAI-1 expression in macrophage during inflammation is not known yet.
Objective: In this study, we investigated the molecular mechanism of PAI-1 expression stimulated by CpG-ODN in macrophages.
Materials & Methods: Reverse Transcription PCR (RT-PCR) and Western blotting were used to monitor the gene expression. Cell surface expression of PAI-1 was analyzed by FACS analysis. Wild-type BALB/c mice and TLR-$9^{-/-}$ BALB/c mice were used to isolate peritoneal macrophages. Promoter activation was monitored by Luciferase assay. Migration of macrophages was monitored in transwell chamber. siRNA mediated knockdown of PAI-1 was performed to confirm the role of PAI-1 in migration.
Results: CpG-ODN treatment significantly enhances the PAI-1 expression through NF-κB-independent pathway in both RAW264.7 cells and mouse peritoneal macrophages. Recognition of CpG-ODN by TLR-9 activates Sp1 and Elk-1 transcription factors, which activates PAI-1 promoter. Activated Sp1 and Elk-1 elevates PAI-1 expression via MEK1/2-ERK and JNK signaling pathways in CpG-ODN-stimulated macrophages. CpG-ODN-induced PAI-1 expression increased the migration of macrophages through vitronectin.
Conclusion: PAI-1 expression is involved in activating MEK1/2-ERK-and JNK-dependent and NF-κB-independent signals and vitronectin-specific migration of macrophages.

Keywords: macrophages, migration, PAI-1, vitronectin, CpG-ODN

Coregulation of the Chemokine Receptor CXCR4 and Growth Factor Receptor IGF-1R Signal Transduction in the Metastatic Breast Cancer Cells

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Introduction: The expression and function of the chemokine G-protein-coupled receptor CXCR4 and insulin-like growth factor 1 tyrosine kinase receptor IGF-1R have been shown to contribute to the metastatic potential of breast cancer cells. Our previous study has demonstrated that transactivation of CXCR4 by IGF-I/IGF-1R is essential for IGF-I-mediated cell migration in the metastatic breast cancer MDA-MB-231 cells.
Objective: The present study was aimed to further examine the interdependence of the two receptors especially in the down-regulation stage of receptor activation in MDA-MB-231 cells.
Materials & Methods and Results: Flow cytometric analysis showed that prolonged stimulation of MDA-MB-231 cells with IGF-I leads to co-internalization of CXCR4 and IGF-1R. Western blot analysis demonstrated that the IGF-I stimulation results in degradation of only IGF-1R but not CXCR4. Incubation of the cells with IGF-I resulted in a significant decrease in the level of calcium mobilization in response to CXCL12, a specific ligand for CXCR4, indicating the reduction of CXCR4 activity. However, retroviral-mediated RNAi knock down of CXCR4 in the cells did not have an effect on IGF-I-induced IGF-1R degradation, suggesting that this process is independent of CXCR4. Inhibitor studies indicated that G-proteins, mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3 kinase (PI3K) are responsible for IGF-I-induced CXCR4 internalization whereas G-proteins and PI3K are involved in IGF-I-mediated IGF-1R degradation.
Conclusion: These findings indicate the coregulation of CXCR4 and IGF-1R by IGF-I in the metastatic breast cancer cells, providing more understanding in the implication of a network of chemokines and growth factors in the metastatic behavior of breast carcinoma.

Keywords: breast cancer, CXCR4, IGF-1R, coregulation

ERα Phenotype, Estrogen Level, and Benzo [α] pyrene Exposure Modulate Tumor Growth and Metabolism of Lung Adenocarcinoma Cells

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Introduction: Women have a higher risk of lung adenocarcinoma than men, suggesting that estrogen pathway may be involved in the pathogenesis of this cancer.
Objective: Whether ERα expression, estrogen levels, and endocrine disruptor exposure would influence tumor growth of lung adenocarcinoma cells.
Materials & Methods: Mice were divided into three groups. One group remained ovary-intact. The other two groups were ovariectomized. E2 was replenished to one group of ovariectomized mice. Tumor cells were inoculated 10 days. Mice were intraperitoneally injected with 0, 0.2, 0.8, and 1.6 mg BaP per week. Tumor cells were inoculated after the third dose of BaP. The first dose of BaP was injected a week after ovariectomy. Tumor cells were inoculated after the third dose of BaP. Tumor volume was measured. Tumors were frozen for gene expression study.
Results: Estrogen promoted tumor growth of ERα$^+$ lung adenocarcinoma cells but inhibited that of ERα$^-$ lung adenocarcinoma cells. Endocrine disruptor benzo[α]pyrene stimulated ERα$^-$ tumor growth dose dependently. Either of ovariectomy and ERα expression abolished the tumor growth-promoting effect of benzo[α]pyrene. Benzo[α]pyrene increased expression of *CYP1B1* over *CYP1A1* and suppressed estrogen-induced *COMT* up-regulation in ER tumor cells, probably switching estrogen metabolism to 4-hydroxyestradiol formation and removing the inhibition of 2-methoxyestradiol on ERα$^-$ tumors. ERα inhibited AhR from up-regulating *CYP1* in response to benzo[α]pyrene exposure, but it increased angiogenic *VEGF-A* expression with body estrogen levels.
Conclusion: Estrogen might increase ERα$^+$ lung adenocarcinoma growth by up-regulating cancer-related ER target gene expression.

Keywords: lung adenocarcinoma, ERα, tumor growth

Analysis of Cellular Proteome and Secretome of Human Endothelial Cells Induced by TNF-α

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Introduction: Patients with severe form of dengue virus infection, dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS), are typically presented with hemorrhage, thrombocytopenia and vascular leakage, together with evidence of elevated serum levels of cytokines or chemokines. TNF-α is one of the key inflammatory cytokines that plays an important role in membrane permeability changes of vascular endothelial cells. However, effects of TNF-α on human endothelial cells at the molecular levels remained poorly understood.
Objective To characterize changes in cellular proteome and secretome of human endothelial cells after induction with TNF-α.
Materials & Methods: Proteins from cell lysate and culture supernatant of human endothelial cells (EA.hy926) with or without TNF-α treatment were resolved by two-dimension polyacrylamide gel electrophoresis (2-DPAGE) and visualized by Deep Purple fluorescence dye. Quantitative intensity analysis was performed using Image Master 2D platinum software and the altered proteins were identified by mass spectrometry.
Results: Proteomic analysis revealed 9 and 10 proteins with increased levels, whereas 3 and 7 other proteins were decreased in the cellular proteome and secretome, respectively, by TNF-α treatment. These altered proteins were functionally classified and most of the altered cellular proteins were involved in transcription/translation regulation, protein transport, metabolism, signal transduction, structure, and protein transport. The altered secreted proteins were involved mainly in metabolism, structure, adhesion, protein modification, anticoagulation, and protein transport.
Conclusion: The altered proteins in human endothelial cells induced by TNF-α were involved in biological functions that might reflect their effects on membrane structure.

Keywords: proteome, secretome, endothelial cells, cytokines

Down-regulation of MHC Class I Associated with Antigen Processing Machinery Defects Initiated by Integrated HPV16 during Cervical Cancer Carcinogenesis

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Introduction: Association between high risk human papillomaviruses and host immune responses are play role through multi-step processes during cervical cancer (CXCA) carcinogenesis. Understandings of these factors are carried out and knowledge in clinical use is limited.
Objective: To investigate HPV16 physical status and major histocompatibility complex (MHC) class I expression associated with alteration of antigen processing machinery (APM) as well as clinical outcome of progression during cervical cancer development.
Materials & Methods: A prospective study was monitored in 169 cervical samples. Embedded paraffin samples of MHC class I and APM were studied. The HPV16 physical state was determined using quantitative PCR, indicating for HPV16 E2 and E6 ratio interpretation. The MHC class I and APM alteration as well as clinical outcome were evaluated relevance to HPV16 physical status occurrence.
Results: We showed association between overall MHC class I down-regulation and APM defects. Combination between MHC class I down-regulation and HPV16 physical status were discovered. We found clinical outcome of progression with MHC class I loss of expression associated with APM defects in the integrated HPV16 occurrence during cervical cancer development, significantly.
Conclusion: MHC class I down-regulation associated with APM defect caused by integrated HPV initiation. The HPV16 physical status and MHC class I expression can be applied as an important prognostic markers to predict clinical outcome of progression during cervical carcinogenesis. However, exact mechanisms should be further explored.

Keywords: MHC class I down-regulation, antigen processing machinery, integrated HPV16, cervical cancer carcinogenesis

Palmitic Acid Enhanced Pro-inflammatory Cytokine Expression of THP-1 Cells

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Introduction: Obesity is a worldwide problem that overtaken malnutrition. Level of saturated fatty acids (SFAs), such as palmitic acid (PA), has been found to be correlated with obesity marker. Furthermore, there is low and chronic inflammation in adipose tissue with increased pro-inflammatory cytokines level, released by macrophage and adipocyte. Some studies proposed that SFAs acted as an inducer but still debatable.
Objective: To determine the effects of PA on pro-inflammatory cytokines expression of THP-1.
Materials & Methods: Cells were incubated with PA in various concentration (0, 25, 50, and 100 μM) and time (0, 2, 4, 6, and 8 hours), continued with LPS stimulation 1ng/μl for 4 hours. TNF-α, IL-1β, IL-6, and IL-8 expressions was measured by qRT-PCR.
Results: PA pre-incubation enhanced pro-inflammatory cytokines compared to PA and LPS alone. TNF-α expression reached plateu level at 25μM and 50μM (P<0.01). IL-1β expression was increased in every concentration but not in a dose dependent manner (P<0.01). On the other hand, IL-6 and IL-8 expressions were increased in a dose dependent manner (P<0.05 and P<0.001, respectively). Moreover, all cytokines expressions reached peak point after 2 hours pre-incubation with PA 100 μM. (TNF-α > 2 fold, IL-1β p< 0.01, IL-6 p< 0.05, IL-8 p < 0.001), then started decreasing. Interestingly, their expression started to increase again in 8 hours pre-incubation.
Conclusion: PA incubation prior to LPS enhanced pro-inflammatory cytokines expression even in low concentration. This enhancement effect can be observed in early and late stages. Hence, their mechanism should be further evaluated.

Keywords: obesity, palmitic acid, pro-inflammatory cytokines

The Effect of Estrogen in Cholangiocarcinoma Cell Migration and Metastasis

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Introduction: High estrogen level in cholangiocarcinoma (CCA) patient serum has been reported that correlated to lower patients' survival time and their stimulatory effect also involved in proliferation and invasion in *in vitro* study.
Objective: To identify the expression of estrogen-related metastasis genes and investigate how estrogen affects CCA metastasis by mimicking processes in animal model.
Materials & Methods: KKU-M213 CCA cell line was performed to investigate the effect of estrogen in CCA cell migration and the expression of metastasis-related genes; *MET* and *TIMP4* by real time RT-PCR method. In *in vivo* experiment, GFP-transfected KKU-M213 cells were injected in nude mice subcutaneously.17β-estradiol (E2) was supplemented to E2-treated group mice daily after injection. Mice were sacrificed to collect serum and tissue samples.
Results: E2-treated CCA cell line showed higher migratory capability than untreated. Real time RT-PCR showed increased *MET* expression and reduced the expression of *TIMP4* in E2-treated condition, which corresponded to migration assay. Larger tumor mass at primary site was detected in E2-treated mice, however, none of mice in this group could be detected the metastatic site. In contrary, we could find the intraperitoneal metastasis nodules in control group.
Conclusion: *In vitro* study suggested that E2 could stimulate CCA migration and regulate the expression of some metastatic-related genes. However, this stimulation could not be proved as expected in animal model. This controversial result could be explained in many aspects and need to further investigate.

Keywords: cholangiocarcinoma, estrogen, metastasis

Estrogen-promoted Resistance to Doxorubicin-induced Apoptosis in Breast Cancer Cell Associated with TFF1 Trefoil Protein

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Introduction: TFF1 trefoil protein is an estrogen-regulated secretory protein expressed in breast cancer. It has been reported that TFF1 can protect conjunctival cells from UV-induced apoptosis.
Objective: To demonstrate the role of TFF1 in estrogen-promoted resistance to doxorubicin-induced apoptosis using MCF-7 model.
Materials & Methods: Permanent knockdown of *TFF1* gene in MCF-7 cell had been generated and used to test the sensitivity to doxorubicin treatment in present or absent of 17beta-estradiol. The apoptosis cells were measured by fluorescence staining and flow cytometry method.
Results: The results showed that among the stimulation of apoptosis by doxorubicin, 17beta-estradiol could suppress this process in control but not in *TFF1* knockdown MCF-7 cells. Moreover, it was shown that anti-TFF1 antibody could reverse the anti-apoptotic effect of estrogen in control and recombinant TFF1 could recover *TFF1* knockdown MCF-7 cell death. However, this process could not be inhibited by fulvestrant, an estrogen antagonist. Apoptosis protein array experiment reflected the role of the anti-oxidative enzymes catalase in estrogen and TFF1 modulated apoptosis, which correlated to enzymatic assay.
Conclusion: These phenomena determine the role of TFF1 in estrogen-promoted resistance to apoptosis induced by doxorubicin in MCF-7 breast cancer cell. *TFF1* gene may be a target for enhancing of sensitivity to chemotherapy in breast cancer treatment.

Keywords: TFF1 trefoil protein, estrogen, MCF-7, doxorubucin, apoptosis

The Canonical NF-κB Pathway Governs Mammary Tumorigenesis in Transgenic Mice and Tumor Stem Cell Expansion

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Introduction: The role of mammary tumor epithelial cell (MEC) NF-κB in tumor progression *in vivo* is unknown as murine NF-κB signaling is required for murine survival or normal mammary gland development.
Objective: As NF-κB is expressed widely in multiple distinct cell types which may each contribute independently to the tumor phenotype, we determined the role of mammary epithelial cell NF-κB in ErbB2 mammary tumorigenesis *in vivo* by developing an animal model that permits inducible suppression of NF-κB activity, targeted to the mammary gland.
Materials & Methods: Transgenic mice were generated encoding an ecdysone-regulated stabilized IκBα protein (IκBα SuperRepressor: IκBαSR). These triple transgenics were crossed to mammary-targeted ErbB2 to determine the requirement for NF-κB activity in ErbB2-mediated mammary tumorigenesis *in vivo*.
Results: Inducible suppression of NF-κB in the adult mammary epithelium delayed the onset and number of new tumors. Within similar sized breast tumors, TAM and tumor neoangiogenesis was reduced. In co-culture MEC NF-κB enhanced TAM recruitment. Genome wide expression and proteomic analysis demonstrated IκBαSR inhibited tumor stem cell pathways. IκBαSR inhibited breast tumor stem cell markers in transgenic tumors, reduced stem cell expansion *in vitro*, and repressed expression of Nanog and Sox2 *in vivo* and *in vitro*.
Conclusion: Mammary epithelial cell NF-κB contributes to mammary tumorigenesis.

Keywords: NF-κB, tumorigenesis, mammary

Toll-like Receptor 4-mediated IL-6 Expression in Paclitaxel Resistance of Breast Cancer

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Introduction: Paclitaxel (PTX) is a potential anti-cancer drug for treatment of advanced breast cancer. Toll-like receptor 4 (TLR4) can bind with PTX and has been reported for its impact in PTX resistance in ovarian cancer. However, TLR4-related PTX resistance in breast cancer has never been clarified.
Objective: This study aims to explore role of TLR4 in PTX resistance in breast cancer.
Materials & Methods: *TLR4* and *MyD88* mRNA levels were measured in MCF-7 and MDA-MB231 breast cancer cell lines by real time PCR. TLR4 protein was determined by Western blot and immunocytochemistry. Transient knockdown of TLR4 by *siRNA* was performed to elucidate effect of TLR-4 in PTX responsiveness by measuring cancer cell survival and expressions of pro-tumorigenic cytokines and anti-apoptotic gene.
Results: MDA-MB231 cells had significantly high *TLR4* and *MyD88* expressions compared to those in MCF-7 cells though TLR4 protein level was similar in both cell types. Transient knockdown TLR4 in MDA-MB231 cells revealed the increased in PTX-induced cell death with statistical significance than that in mock cells. The result showed significant more reduction of *IL-6* expression, but not *IL-8* and anti-apoptotic gene XIAP, from siTLR4-treated MDA-MB231 cells after exposure to PTX.
Conclusion: The potential of cancer cell to produce IL-6 through PTX-TLR4 ligation is suggested. Since IL-6 is a pro-tumorigenic cytokine, this effect can partly induce cancer cells to resist to PTX treatment. Though the additional breast cancer cell lines will be utilized to confirm, TLR4 activates IL-6 expression may have impact in PTX resistance in breast cancer. This work is financial supported by Siriraj Graduate Scholarships and Siriraj Graduate Thesis Scholarships. Breast cancer cell lines are kindly donated from Dr. Susan Eccles, UK and Professor Dr. M.R. Jisnuson Svasti, Thailand.

Keywords: Toll-like receptor 4, Paclitaxel, breast cancer, resistance

A Modulation of RANTES Production by Dengue Virus Nonstructural Protein 5 and Daxx Interaction

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Introduction: A remarkably increased production of cytokines is observed in the patients with dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS). Dengue virus nonstructural protein 5 (DENV NS5) has been reported to be involved in a cytokine induction. However, the molecular mechanism by which DENV NS5 mediates these responses has not fully been elucidated.

Objective: This study aims to identify host proteins, which interacts with DENV NS5 and the mechanism, which is involved in increased cytokine production.

Materials & Methods: Yeast two-hybrid assay was performed to identify host proteins interacting with DENV NS5. Wild-type DENV NS5 and DENV NS5-K/A, which were mutated at the nuclear localization sequences (NLS), were expressed in HEK293 cells to assess its roles in nuclear translocation, host protein interaction, and cytokine production. Co-immunoprecipitation and co-localization assays were performed to confirm the *in vivo* relevance of this interaction. Real-time RT-PCR and ELISA were performed to measure the amount of cytokine production in mRNA and protein levels, respectively.

Results: A death domain-associated protein (Daxx) was identified to interact with DENV NS5 by yeast two-hybrid assay. In HEK293 cells, Daxx interacted and nuclear co-localized with wild-type DENV NS5. This interaction was associated with the increasing of the DHF-associated cytokine, RANTES (CCL5) production. In the absence of NLS (DENV NS5-K/A), DENV NS5 could neither translocate into the nucleus nor interact with Daxx to increase RANTES production.

Conclusion: This work demonstrates a modulation of RANTES production by DENV NS5 and Daxx interaction.

Keywords: dengue virus, NS5, Daxx, RANTES, CCL5

Synergistic Antitumor Activity of mTOR Inhibitor (RAD001) and HDAC Inhibitors (LBH589, MS275 and Salermide) on Pancreatic Cancer Cell Line BxPC-3

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Introduction: Treatments targeted against cellular signalling pathways have shown promise in the management of solid tumors and hematological malignancies. The PI3K/AKT/mTOR signaling axis is a critical mediator of cancer-cell survival and resistance. Combinations of agents with different antitumor mechanisms are desired to maximize efficacy. For this purpose, histone deacetylases (HDACs), which regulate gene expression involved in cancer cell differentiation and apoptosis, have been shown to be relevant targets and various inhibitors of histone deacetylases (HDACis) are in clinical development.
Objective: We aimed to investigate the effect of RAD001 plus chemotherapeutic agents HDAC inhibitors (LBH589, MS275 and Salermide) on proapoptotic and proliferative gene expressions.
Materials & Methods: BxPC-3 cells (pancreatic cancer cell line) were treated with mTORC1 inhibitor (RAD001), and HDACis (i.e. LBH589, MS275 and Salermide). Effects of agents on cell viability were assessed using MTT assay. Moreover, expression levels of the genes regulating cell cycle and proliferation were evaluated using quantitative Real Time PCR. Changes on global histone acetylation levels were also determined by ELISA assay.
Results: The combinations of RAD001 with HDAC inhibitors (e.g., LBH589, MS275 and Salermide) were found to decrease cell viability more than each individual agent alone. Moreover, the effects of combined and single agent treatment on BxPC-3 cells were also studied at mRNA gene expression level.
Conclusion: We have demonstrated the synergistic antitumor activity achieved by combining RAD001 with HDACis in BxPC-3 cells *in vitro*. Further studies are required to verify the molecular mechanisms by which blockade of mTOR signaling and HDAC activity affects chromatin remodeling.

Keywords: HDAC inhibitors, RAD001, pancreatic cancer cell line

Investigation of Apoptotic and Anti-proliferative Effects of Akt and ERK1/2 Inhibitors in Colon Cancer Cell Lines

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Introduction: Colon cancer is the most common gastrointestinal cancer in the world. PI-3K/Akt and Ras/Raf/MEK/ERK signal transduction is found to be impaired and overexpressed in many types of cancer. While FR180204 is a selective inhibitor of ERK1 and ERK2, API-1 is a selective inhibitor of Akt signaling.
Objective: We aimed to the changes in mRNA and some protein expression levels of Bax, Bak, Bim, CycD, c-myc, Bcl-2, Bcl2L1 and FOXO3A and investigate the apoptotic, cytotoxic and anti-proliferative effects of API-1 and FR180204 (FR) seperately and combined treatment on DLD-1 and LoVo cell lines.
Materials & Methods: The effects of two agents on cell viability, DNA synthesis rate, cytotoxicity, Relative mRNA and protein expression levels were analyzed using XTT, BrdU-ELISA, LDH release assays, Real-time PCR and Western blotting method respectively.
Results: FR administration decreased cell viability in time and dose dependent manner in both cell line. Hovewer API treatment alone or combination with FR decreased cell viability until a particular concentration in DLD-1 cells. DLD-1 cells were affected lower than LoVo cells by FR and API-1 administration. In addition, this combination treatment reduced DNA synthesis rate much more than either agents did alone. Real time PCR analysis showed that Bcl-2, Bcl-XL, CycD and c-myc mRNA levels were down-regulated and Bax, Bak, FOXO3A mRNA levels were up-regulated after FR and API-1 and these results were confirmed with protein levels.
Conclusion: According to the results of this study, we believe that it may be fundamental to further evaluate this inhibitory drug interactions in colon cancer.

Keywords: colon cancer, apoptosis, ERK1/2, Akt, API-1, FR180204

Development of a Novel Protease Sensor Using an Engineered Autoinhibited Protein

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Introduction: Proteases are involved in nearly every biological process, and thus the abnormal activity of them is an important indicator of disease. Various methods have been developed to analyze protease activity, but there is still a need for new methods with improved sensitivity. In this presentation, we describe a novel protease sensor using an engineered autoinhibited protein.
Objective: The aim of this study is to develop a highly sensitive protease assay method using an autoinhibited protein.
Materials & Methods: MMP2 and caspase-3 were purchased from R&D systems, Inc., and the sensor proteins developed in this study were expressed *in Escherichia coli* and were purified using a metal affinity chromatography.
Results: An autoinhibited protein is designed to develop a protease assay method. The protein includes activity domain (A) and inhibitory domain (I), and the two domains are connected via a protease-cleavable sequence. A cleavage reaction in the linker removes the intramolecular interaction, and the activity domain can show a measurable signal coupled with a detection method, in this study ELISA. We applied the developed method for two proteases, MMP2 and caspase-3. The detection limits for MMP2 and caspase-3 were as low as 3 and 0.05 ng/mL respectively.
Conclusion: We have developed a novel method for protease activity and applied the method for two clinically relevant proteases, MMP2 and caspase-3. The design strategy for the autoinhibited protein is modular, and the approach can be easily modified for other proteases by replacing the protease cleavable site.

Keywords: protease assay, sensor, autoinhibited protein

Determination of the EF-24's Effect on Taxol's Apoptotic and Anti-proliferative Response in HeLa Cell Line

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Introduction: Cervical cancer is the third most common malignancy in women worldwide, and it remains a leading cause of cancer-related death for women in developing countries. Taxol is used as chemotherapeutic agent in various cancer types. EF-24, one of the curcumin's synthetic analogues, is used in a variety of cancer studies.
Objective: In this study, we aimed to research the apoptotic or anti-proliferative effect of Taxol and EF-24 on HeLa cells.
Materials & Methods: In our study, after Taxol and EF-24 were implemented on HeLa cell line both individually and combined, cell viability and relative mRNA expression levels were analyzed using MTT and Real-time PCR method, respectively.
Results: In our study, we observed that the viability of cells decreased when EF-24 and Taxol were applied separately. However, EF-24 treatment alone decreased cell viability much more than combined treatment of EF-24 and Taxol. These results showed that this agent exhibited an antagonistic effect. Combination with EF-24 and low dose Taxol increased the effect of Taxol, but with high concentration of Taxol we didn't see the same effect. When we applicated those drugs together, analysis of mRNA levels of caspase-3, caspase-9, cyclin D1 and PI3K also showed the similar results.
Conclusion: Further investigation of EF-24 and its combination with Taxol or other chemotherapeutics are needed to give us more effective opportunity for the treatment of cervical cancer.

Keywords: gene expression, apoptosis, cervical cancer, taxol, EF-24

p38 MAP Kinase Regulates Cyclopamine-induced Apoptosis of Erythroleukemia Cells in Association with COX-2 Overexpression

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Introduction: Cyclooxygenase-2 (COX-2) has been described to play a crucial role in the proliferation and differentiation of leukemia cells. Furthermore, several studies reported a role for this enzyme in chemoresistance in different cancer types. Previously in our laboratory, we showed an activation of the metabolism of arachidonic acid in apoptotic process and differentiation induced by diosgenin, a plant steroid, resulting in COX-2 activation.
Objective: For the first time, we focused on the effect of cyclopamine and jervine, two steroidal alkaloids, on apoptosis induction and COX-2 expression in human erythroleukemia cell lines.
Materials & Methods: We used MMT assay for proliferation test, JC-1 staining for mitochondrial membrane potential analysis, DNA fragmentation for apoptosis quantification, western blot for COX-2 expression and enzyme immunoassay for prostaglandin E2, electromobility shift assay for nuclear factor-κB (NF-κB) activation and ELISA kit for specific quantitation of each mitogen-activated protein kinase (MAPK).
Results: Despite their close structures, we showed that cyclopamine but not jervine induced apoptosis in HEL and TF1a human erythroleukemia cells. Both cyclopamine and jervine induced COX-2 overexpression but through distinct pathways. In cyclopamine-treated cells, this overexpression was p38 MAP kinase (pro-apoptotic pathway) dependent. Inhibiting the p38 pathway led to COX-2 inhibition and an increase of apoptosis. However in jervine-treated cells, COX-2 overexpression was dependent on NF-κB activation (anti-apoptotic pathway). Inhibition of this pathway led to COX-2 inhibition and apoptosis induction.
Conclusion: Altogether, these results suggest a role for COX-2 in resistance to apoptosis in HEL and TF1a human erythroleukemia cells.

Keywords: cyclopamine, apoptosis, cyclooxygenase-2, cancer

Development of Novel Diagnostic System towards Cancer Using PKC Delta

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Introduction: Protein kinase C delta plays a major role in progression of human brain tumors and expression of outer membrane antigen CD133 known as cancer stem cell biomarker. Till date, there is no effective diagnostic system targeting the PKCδ. In this study, we develop the detection system for potent cancer stem cell biomarker PKCδ and evaluated their inhibitory role.
Objective: Development of novel diagnostic system using peptide by phage display for the early detection of cancer stem cell biomarker PKCδ.
Materials & Methods: We used an M13 phage display library to screen for peptides that specifically bind PKCδ protein. *Escherichia coli* strain, Rosetta (DE3), was used as expression host to produce PKCdelta catalytic domain protein.
Results: We found two peptides from the peptide library of size $2.7*10^9$, which showed higher affinity and inhibitory role towards their target PKC.
Conclusion: The found peptides against PKCδ with higher affinity and inhibitory role would be further useful in developing novel biosensor based diagnostic system in the detection of cancer at early stages.

Keywords: cancer stem cell, phage display, peptide, PKCδ

Novel Transcription Targets and Chemical Inhibitors of PAX3-FKHR for the Development of Therapy to Treat Rhabdomyosarcoma

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Introduction: More than 80% of alveolar rhabdomyosarcoma (ARMS), a childhood sarcoma with a 5-year survival rate of less than 30%, harbor a PAX3-FKHR fusion transcription factor, which regulates cell migration and promotes metastasis, possibly by regulating PAX3-FKHR's transcription targets. Identifying druggable transcription targets of PAX3-FKHR may lead to novel therapeutic approaches, and discovery of chemical inhibitors of PAX3-FKHR or its downstream effectors may lead to the development of effective therapy for ARMS.
Objective: To identify transcription targets and downstream effectors, and chemical inhibitors of PAX3-FKHR.
Materials & Methods: We developed an ARMS cell line in which PAX3-FKHR is down-regulated, resulted in significant reduction in cell motility. We used microarray to identify genes downregulated in this cell line; mutational analysis, promoter reporter and gel shift assays to determine whether PAX3-FKHR binds to target gene's promoter; siRNA, pharmacologic inhibitor, or recombinant protein to modulate target gene's levels and investigated the effect on cell motility; high-throughput screening (HTS) to identify inhibitors of PAX3-FKHR.
Results: Among the genes identified, carnitine palmitoyltransferase 1A (CPT1A) and pleiotrophin (PTN) harbor binding sites for PAX3-FKHR in their promoter. Downregulating CPT1A or PTN decreased cell motility in ARMS cells. HTS identified natural compounds that modulate PAX3-FKHR's post-translational modification, downregulate the expression of CPT1A and PTN, and decrease ARMS cell invasion and anchorage-independent growth.
Conclusion: CPT1A and PTN are novel transcriptional targets and downstream effectors of PAX3-FKHR that may serve as biomarker and novel therapeutic targets for the treatment of ARMS. Chemical inhibitors of PAX3-FKHR may be developed as therapeutics for ARMS.

Keywords: pediatric cancer, high throughput screening, post-translational modification, natural compounds, gene transcription

Personalized Paclitaxel Therapy for Cancer

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Introduction: Personalized medicine consists of two components: 1) PK (pharmacokinetics) component where the drug dose is optimized to the patient and 2) the PD (pharmacodynamics) component where the treatment modality is matched to that of the patient.
Objective: To personalized paclitaxel therapy, we developed point-of-care (POC) testing devices for PD biomarkers (CA125, BNP, FSH, LH, hCG) and for paclitaxel PK.
Materials & Methods: Serum samples collected at time of diagnosis of ovarian cancer were tested using rapid and quantitative POC devices for blood biomarkers (CA125, FSH, BNP, LH, hCG and paclitaxel) and the data were evaluated using JMP9 statistical analysis software.
Results: 41 ovarian cancer patients were analyzed for CA125 prior to surgery of which 3 (7%), 11 (27%), and 27 (66%) were clear cell carcinoma, cystadenocarcinoma and adenocarcinoma, respectively. CA125 was not diagnostic of cancer. Incidence of elevated BNP (> 25 pg/ml) was higher for patients (14 of 19, 74%) vs. normal controls (3 of 10, 30%, p = 0.02, Chi-square). FSH level was higher (median=151.6 mU/ml) vs. normal controls (median of 13.4 mU/ml, p = 0.01, Wilcoxon). No differences were detected for LH and hCG. Paclitaxel device was able to monitor paclitaxel levels as low as 20 ng/mL.
Conclusion: Quantitative POC tests for personalized paclitaxel therapy have been developed. These tests should allow for more effective dosing of the patients and thereby improving effectiveness of therapy. These POCTs are also patient-centric, inviting better compliance and patient participation in personalizing his/her treatment. Clinical testing will be attempted within the coming year.

Keywords: paclitaxel, personalized therapy, pharmacokinetics

Sensitization of Human Carcinoma Cells by Human Serum Albumin-coated Liposomal Bcl-2 Antisense Oligodeoxyribonucleotide to Chemotherapeutic Drug, Doxorubicin

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Introduction: Cancer is one of the most deadly diseases. Gene-based therapy is a new approach that selectively targets cancer cells, while reducing toxicity on normal cells. Bcl-2 protein is a target for cancer gene therapy, which is overexpressed in cancer cells and exhibits antiapoptotic activity.
Objective: This study aimed to examine the carcinoma cell sensitization induced by human serum albumin-coated liposomal bcl-2 antisense oligodeoxyribonucleotide before treating with chemotherapeutic drug, doxorubicin.
Materials & Methods: Human serum albumin-coated liposomal bcl-2 antisense oligodeoxyribonucleotide was evaluated for bcl-2 downregulating activity, cell growth inhibition, and chemosensitization of KB human oral carcinoma cells to doxorubicin.
Results: Treatment of the cells with human serum albumin-coated liposome-oligodeoxyribonucleotide complexes resulted in bcl-2 mRNA and protein downregulation. Human serum albumin-coated liposome-oligodeoxyribonucleotide complexes inhibited cell growth at the antisense oligodeoxyribonucleotide concentration of 0.45-7.2 μM. Upon post-treatment with doxorubicin, the cytotoxicity was further increased. The cytotoxicity was dependent on antisense oligodeoxyribonucleotide concentration, incubation time and doxorubicin concentration.
Conclusion: Bcl-2 antisense oligodeoxyribonucleotide delivered with human serum albumin coated liposomes reduced the growth of KB oral carcinoma cells and increased the chemosensitivity to doxorubicin.

Keywords: antisense oligodeoxyribonucleotide, Bcl-2, chemosensitization, doxorubicin, liposome

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Suberoylanilide Hydroxamic Acid, an Inhibitor of Histone Deacetylase, Enhances Radiosensitivity and Suppresses Lung Metastasis in Breast Cancer *in vitro* and *in vivo*

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Introduction: Breast cancer is the major cause of cancer-related deaths of women worldwide. Breast cancer treatment involves surgery, chemotherapy, or radiation therapy. Unfortunately, the efficacy of conventional therapy is limited. Thus, novel strategies are needed to boost the oncologic outcome.
Objective: The aim of this study was to examine whether suberoylanilide hydroxamic acid (SAHA), an inhibitor of histone deacetylase, could enhance radiosensitivity and suppress lung metastasis in breast cancer.
Materials & Methods: Radiosensitization was analyzed by a clonogenic assay. Flow cytometry was used to determine apoptosis and autophagy. The ultrastructures of 4T1 cells were observed by EM microphotography. Western blotting was applied to determine the protein expression related to endoplasmic reticulum (ER) stress and autophagy. BALB/c nude mice were orthotopically implanted with 4T1 cells treated with ionizing radiation (IR) or SAHA alone or in combination. The tail vein assay of lung metastasis was used to examine the antimetastatic effects of SAHA *in vivo*. The growth of tumors were measured with the IVIS Imaging System.
Results: IR combined with SAHA showed increased therapeutic efficacy. The combined treatment enhanced ER stress and autophagy. In an *in vivo* study, the combination treatment showed greater anti-tumor growth effects. SAHA inhibited matrix metalloproteinase-9 expression and activation. Our results also indicated that SAHA inhibited the lung metastasis of 4T1 cells *in vivo*.
Conclusion: The data suggest that SAHA enhances radiosensitivity and suppresses lung metastasis in breast cancer *in vitro* and *in vivo*. SAHA could be a new potential therapeutic strategy for the treatment of breast cancer.

Keywords: breast cancer, radiosensitivity, metastasis, autophagy, endoplasmic reticulum stress

The Effectiveness of Cucurbitacin B in BRCA1 Defective Breast Cancer Cells

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Introduction: Cucurbitacin B (CuB) is one of the potential agents for long term anticancer chemoprevention. Cumulative evidences has shown that cucurbitacin B provides potent cellular biological activities such as hepatoprotective, anti-inflammatory and antimicrobial effects, but the precise mechanism of this agent is not clearly understood.
Objective: We examine the biological effects on breast cancer cells of cucurbitacin B extracted from a Thai herb, *Trichosanthes cucumerina* L.
Materials & Methods: The wild type, mutant and knocked-down BRCA1 breast cancer cells were treated with the cucurbitacin B and determined for the inhibitory effects on the cell proliferation, migration, invasion, anchorage-independent growth. The gene expressions in the treated cells were analyzed for $p21/^{Waf1}$, $p27^{Kip1}$ and survivin.
Results: Our previous study revealed that loss of BRCA1 expression leads to an increase in survivin expression, which is responsible for a reduction in sensitivity to paclitaxel. In this work, we showed that cucurbitacin B obviously inhibited knocked-down and mutant BRCA1 breast cancer cells rather than the wild type BRCA1 breast cancer cells in regarding to the cellular proliferation, migration, invasion and anchorage-independent growth. Interestingly, cucurbitacin B promotes the expression of $p21/^{Waf1}$ and $p27^{Kip1}$ but inhibit the expression of survivin.
Conclusion: Cucurbitacin B could effectively inhibit BRCA1 defective breast cancer cells properties. We also suggest that survivin could be an important target of cucurbitacin B in BRCA1 defective breast cancer cells.

Keywords: BRCA1, cucurbitacin B, survivin, $p21/^{Waf1}$, $p27^{Kip1}$

The Mu Opioid Receptor Is a Novel Therapeutic Target for Human Lung Cancer

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Background: Recent epidemiologic studies suggesting differences in cancer recurrence contingent on anesthetic regimens have raised the possibility that mu opioid agonists can influence cancer progression. Based on our previous observations that lung cancer progression is inhibited in mu opioid receptor (MOR) knockout mice and by silencing MOR expression in lung cancer cells, this study examined whether MOR antagonism could be utilized as a novel therapeutic strategy for human non-small cell lung cancer (NSCLC).
Materials & Methods: We utilized shRNA, overexpression vectors as well as treatment with the peripheral MOR antagonist, methylnaltrexone (MNTX) in human H358 NSCLC cells for in vitro (proliferation, migration, invasion, epithelial mesenchymal transition (EMT)) and *in vivo* (tumor growth and metastasis in nude mouse xenografts) assays. Cells were either untreated or treated with various concentrations of MNTX, DAMGO, morphine, fentanyl, EGF or IGF and biochemical and function assays were performed.
Results: Our results indicate MOR regulates opioid and growth factor-induced EGF receptor signaling (Src, Gab-1, PI3K, Akt and STAT3 activation), proliferation, migration and EMT, effects that were inhibited by MOR shRNA or MNTX. In addition, MOR overexpression increased human lung cancer tumor growth and metastasis.
Conclusion: Our data suggests a possible direct effect of MOR on opioid and growth factor signaling and consequent proliferation, migration and EMT transition during lung cancer progression. Further, MOR overexpression promotes human lung cancer tumor growth and metastasis. These effects provide a plausible explanation for the epidemiologic findings. Our observations suggest that MOR antagonism in NSCLC merits further study as a therapeutic option.

Keywords: lung cancer, mu opioid receptor, growth factor receptor signaling, metastasis

Zebularine Inhibits Tumorigenesis and Stemness of Colorectal Cancer *via* p53-dependent Endoplasmic Reticulum Stress

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Introduction: Aberrant DNA hypermethylation is frequently found in tumor cells. Inhibition of DNA methylation reactivates tumor suppressor genes and makes it an effective anticancer strategy.
Objective: In this study, the therapeutic effect of DNA methyltransferase (DNMT) inhibitor zebularine (Zeb) on colorectal cancer (CRC) was investigated.
Materials & Methods: The *in vitro* anticancer activity was investigated mainly in human CRC cell lines, and *in vivo* anticancer activity was evaluated by tumor xenografts and mouse colitis-associated CRC models. Molecular mechanisms were investigated using cDNA microarray analysis and various cell assays. The expression level of several important proteins was examined in CRC patient specimens and HCT116-derived colonospheres.
Results: Zeb exhibited anticancer activity in cell cultures, tumor xenografts and mouse colitis-associated CRC model. Zeb induced p53 stabilization through DNA damage and ribosomal protein S7 (RPS7)/MDM2 pathways. Zeb-induced cell death was dependent on p53 and partially p21. Microarray analysis revealed that genes related to endoplasmic reticulum (ER) stress and unfolded protein response (UPR) were affected by Zeb. Western blot analysis showed that Zeb induced p53-dependent ER stress and autophagy. Pro-survival markers of ER stress/UPR (GRP78) and autophagy (p62) were increased in tumor tissues of CRC patients and HCT116-derived colonospheres. Zeb could downregulate GRP78 and p62, and upregulate a pro-apoptotic CHOP.
Conclusion: Our results reveal a novel mechanism for the anticancer activity of Zeb, and provide a rationale for the clinical application of Zeb in CRC.

Keywords: colorectal cancer, DNA methyltransferase inhibitors, endoplasmic reticulum stress

Anti-cancer and Anti-angiogenesis of a Novel Soluble Beta-glucan in HepG2 Cells-implanted Nude Mice

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Introduction: β-glucans (BGs) are naturally occurring glucose polymers found in yeasts, fungi, mushrooms, bacteria and plants. BGs have been gaining interest due to their multiple functions and therapeutic properties of the derived bioactive.
Objective: To investigate an anti-cancer and anti-angiogenic activity of our novel soluble BG (SBG) in the hepatocellular carcinoma (HepG2)-implanted mice.
Materials & Methods: The cytotoxic effects of SBG on HepG2 cell line were tested by MTT assay. Anti-angiogenic activity of SBG was examined by HepG2-implanted nude mice model. One month after HepG2 injection, the experimental groups were daily oral fed with SBG (16 mg/kg BW). One month post-treatment, the capillary density (CD) was assessed by counting protein expression of CD31-positive cells per field (0.4 mm^2). Apoptotic cells in HepG2-implated tissue were visualized by using TUNEL method. Tumor volume was also determined.
Results: IC50s were 13,144.31 and 7,821.44 μg/ml, after incubation with SBG at 24 and 48 hrs, respectively. The CD and tumor volume in HepG2+vehicle group were 42±3.65 cell/0.4 mm^2 and 348.96±24.97 mm^3, respectively. The treatment with our novel SBG markedly attenuated CD (42±3.65 vs 14±2.99 cell/0.4 mm^2, $P<0.001$) and tumor volume (123.37±6.83 vs 348.96±24.97 mm^3, $P<0.001$). Interestingly, the treatment with SBG induced apoptosis when compared to a vehicle group.
Conclusion: SBG markedly decreased the tumor growth and tumor angiogenesis associated with apoptosis induction in HepG2-induced tumor in nude mice model. Our data suggest a potential therapeutic role of SBG in the tumor progression treatment.

Keywords: soluble β-glucan, hepatocellular carcinoma, CD31, apoptosis

In vitro Anti-inflammatory Activity of JJSK14, a Newly Synthesized Diarylmethylamine Derivative

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Introduction: Overproduction of nitric oxide (NO) generated by inducible nitric oxide synthase (iNOS) and prostaglandin E_2 (PGE_2) generated by cyclooxygenase-2 (COX-2) play a critical role in inflammation-related diseases such as cancer, Alzheimer's disease, rheumatoid arthritis and septic shock. In an effort to discover a novel anti-inflammatory agent, we synthesized newly diarylmethylamine derivatives. The compound JJSK14, *tert*-butyl (2,4,6-trimethoxyphenyl) (cyclopentyl) methylcarbamate showed the most potent anti-inflammatory effect in lipopolysaccharide (LPS)-induced RAW 264.7 macrophage model.
Objective: To study the anti-inflammatory activity of JJSK14 and mechanism underlying its activity on LPS-induced macrophages.
Materials & Methods: Nitrite and PGE2 concentration in conditioned media of 264.7 macrophages treated with LPS plus JJSK14were determined by Griess reaction and ELISA. Cell viability was monitored by MTT assay. iNOS and COX-2 protein levels as well as NF-κB activation were examined by Western-blot analysis. Determination of mRNA level was performed by real time RT-PCR.
Results: We found that JJSK14 has an inhibitory effect on NO production (IC_{50} = 23.77 μM) and PGE_2 production (IC_{50} = 21.98 μM) in LPS-stimulated macrophages without cytotoxic effect. JJSK14 attenuated the expression of iNOS and COX-2 but failed to affect the iNOS enzymatic activity. Additionally, JJSK14 had no effect on the nuclear translocation of NF-κB.
Conclusion: These results indicate that JJSK14 exerts inhibitory activity on NO and PGE2 production through suppression of iNOS and COX-2 expression via a signaling pathway that does not involve NF-κB nuclear translocation. Thus, JJSK14 has potential to be used as a novel compound for the treatment of inflammatory diseases.

Keywords: diarylmethylamine derivative, macrophage, anti-inflammatory activity, nitric oxide, prostaglandin

Bortezomib, a Proteasome Inhibitor, Induces Apoptotic and Autophagic Cell Deaths of Cholangiocarcinoma

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Introduction: Cholangiocarcinoma (CCA) is an aggressive tumor. It poorly responds to conventional chemotherapy. Recent advances of targeted therapy are enlightening CCA treatment. The inhibition of proteasome is of our interest.
Objective: This study was aimed to investigate antitumor efficacy of the proteasome inhibitor, bortezomib, against CCA.
Materials & Methods: Liver-fluke associated CCA cell lines were used. Cell proliferation, cell cycle distribution and apoptosis detection were determined by a tetrazolium-based assay, propidium iodide and annexinV staining, respectively. Evidences of endoplasmic reticulum (ER) stress and the autophagosome formation were demonstrated by Western blot and cytofluorescent detection. The *in vivo* antiproliferative effect was accessed in a subcutaneous transplantation mouse model.
Results: Bortezomib inhibited growth of all CCA cell lines tested in a dose- and time-dependent manner. Induction of G2/M cell cycle arrest and apoptosis were observed. Accumulations of proteins leading to ER stress response and autophagy induction were detected. The growth inhibitory effect of bortezomib was supported by xenograft transplantation outcomes.
Conclusion: Effects of bortezomib in induction of apoptotic and autophagic cell deaths in CCA were evident in the present study. This could be a promising therapeutic approach to the treatment of CCA.

Keywords: cholangiocarcinoma, bortezomib, targeted therapy, apoptosis, autophagy

Isorhamnetin Inhibits the Growth of Human Gastric Cancer and Chemosensitizes It to Capecitabine in a Xenograft Mouse Model through the Modulation of NF-κB Pathway

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Introduction: Gastric cancer (GC) is the second-most common cause of cancer-related deaths. Because of poor prognosis and development of chemoresistance, the existing treatment modalities for GC are ineffective, requiring for safe and effective novel agents.
Objective: Whether isorhamnetin (IH), a 3'-O-methylated metabolite of quercetincan sensitize gastriccancer to capecitabine in a xenograft mouse model was investigated.
Materials & Methods: The effect of IH on proliferation of GC cell lines was analyzed by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay, on NF-κB (nuclear factor kappa B) activation by DNA binding assay and on various gene products by western blot analysis. The effect of IH on tumor growth and on chemosensitization was examined using subcutaneously implanted tumors in nude mice.
Results: IH inhibited the proliferation of various gastric cancer cell lines, potentiated the apoptotic effects of capecitabine, inhibited the constitutive activation of NF-κB and modulated the expression of the NF-κB regulated gene products in GC cells. The xenograft GC model showed that administration of IH alone (1 mg/kg body weight, i.p. thrice/week) significantly suppressed tumor growth which was further enhanced by capecitabine. As compared to vehicle control, IH could also suppress NF-κB activation and alter the expression of cyclin D1, p53, COX-2 (cyclooxygenase-2), MMP-9 (Matrix metallopeptidase 9) and Bcl-xL (Bcl-2 like protein 1).
Conclusion: Overall our results demonstrate that isorhamnetin can potentiate the effects of capecitabine through the suppression of NF-κB regulated markers of proliferation, invasion, and angiogenesis.

Keywords: gastric cancer, isorhamnetin, chemoresistance, proliferation, NF-κB

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Anticancer Efficacy of Squalenoyl Cisplatin Nanomedicine on Human Colonic Cancer Cells

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Introduction: Colorectal cancers constitute a leading cause of death in Western countries. Cisplatin and Oxaliplatin (a third generation platinum antitumor compound) are the most commonly used chemotherapeutic agents; they are prescribed as a first line therapy for most malignancies, including colon cancer in association with 5-Fu. Their clinical use is, however, dose limited due to systemic toxicity, primarily to the kidney. To overcome these drawbacks and to improve the therapeutic index of cisplatin, we have recently developed the concept of squalenoylation which consisted in the bioconjugation of cisplatin with squalene, a natural and biocompatible triterpene. Remarkably, the resulting bioconjugates were able to self-assemble spontaneously as nanoparticles that were narrowly distributed in 100–140 nm size range.
Objective: The current study aims to evaluate the anticancer efficacy of cisplatin-loaded nanoparticles (SQCDDP).
Materials & Methods: The cytotoxic/proapototic activities of SQCDDP on the human colonic cell lines HT29 and KM12 were assessed using phase contrast microscopy, biochemical and immunohistochemical approaches.
Results: This *in vitro* evaluation has allowed to demonstrate that SQCDDP affected the growth of HT29 and KM12 cells at concentrations (IC50 0.6±0.21μM and 1.5±0.78 μM for HT29 and KM12, respectively) ten times lower than cisplatin. This process involved a caspase 3/7-dependent apoptotic pathway. The molecular mechanisms underlying the anticancer activity of SQCDDP are under investigation.
Conclusion: Squalenoyl cisplatin nanomedicine displayed an increased antitumor efficacy on human cancer cells. This opens up the possibility to increase the maximal tolerated dose of cisplatin, thereby validating the potential of nanotechnology to impact global health.

Keywords: cisplatin, nanomedicine, squalenoylation, colorectal cancers, apoptosis

11-Hydroxy and Rostenedione Metabolism in Prostate Cancer Cells

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Introduction: Dihydrotestosterone (DHT), the most potent natural androgen, plays a central role in prostate cancer. Metastatic tumors often progress to DHT-dependent castration-resistant prostate cancer (CRPC) even though androgen deprivation therapy depletes testosterone (T), the DHT precursor. In CRPC, DHT is produced from adrenal androgens: dehydroepiandrosterone (DHEA), androstenedione (A4) and T. We recently showed that the adrenal, however, also produces 11-hydroxyandrostenedione (11OH-A4) by the hydroxylation of A4, catalysed by P45011β-hydroxylase (CYP11B1).
Objective: Adrenal androgen metabolism in normal and prostate cancers cells and key metabolite interaction with the androgen receptor (AR) were investigated.
Materials & Methods: T and 11OH-A4 production levels were compared in the H295R adrenal cell model. T and 11OH-A4 conversion was assayed in COS-1 cells expressing 5α-reductase type 1 and 2. T and 11OH-A4 metabolism was investigated in prostatecancer cell lines. DHT, 11OH-A4 and 11β-OH-5α-androstanedione (11OH-5α-dione) were assayed in COS-1 cells expressing the AR and an ARE-luciferase promoter reporter construct.
Results: H295R cells produce significantly higher levels of 11OH-A4 (90.1nM) compared to T (39.2 nM). Forskolin stimulation increased 11OH-A4 and T levels 4.3- and 1.2-fold, respectively. 5α-reductase type 1 and 2 converted 11OHA4 to 11OH-5α-dione. UPLC-MS/MS analyses of 11OH-A4 metabolites identified DH11OH-A4 and 11keto-A4 in LNCaP cells. At 10 nM, activation by DHT, 11OH-A4 and DH11OH-A4 was 59-fold, 6-fold and 47-fold, respectively.
Conclusion: The adrenal produces significantly higher levels of 11OH-A4 than T. In cancer prostate cells, 11OH-A4 is converted to DH11OH-A4. Activation of the AR suggests that DH11OH-A4 may be an active androgen in target tissue, indicating possible clinical strategies to include CYP11B1 inhibition.

Keywords: adrenal androgens, 11-hydroxyandrostenedione, 5α-dione, prostate cancer, DHT

Geldanamycin Inhibits Cell Growth and Induces Apoptosis in Osteosarcoma 143B Cell Line

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Introduction: Heat shock proteins (Hsps) belong to chaperones that are responsible for maintaining homeostasis of the organisms and promoting cell survival induced by various chemical and physical factors. Geldanamycin (GA) has been reported by numerous studies to possess anticancer properties mainly associated with its inhibition of Hsp90 activity.
Objective: The purpose of this study involved determining the anticancer effectiveness and mechanism of action of GA in highly metastatic osteosarcoma 143B cell line.
Materials & Methods: Inhibition of cell growth was investigated by MTT assay, induction of apoptosis by flow cytometry, protein level by Western blot and immunofluorescence methods, while gene expression by Real Time PCR.
Results: We noticed that the antiproliferative effect of GA was concentration dependent with an EC50 value of 1.3 ± 0.16 μM. After 24 h incubation with 2 and 4 μM GA the level of apoptotic cells was increased to 12% ± 1.72 and 31.5% ± 3.46, respectively. Moreover, we observed that treatment with GA resulted in downregulation of gene expression and protein level of Hsp90, while upregulated Hsp70. Grippingly, GA did not affect on Hsp60 gene expression, but probably determined Hsp60 protein modification.
Conclusion: Our data demonstrated that GA through disrupting multiple oncogenic signaling pathways (involving regulation of Hsp60, 70, 90 gene expression and protein levels) resulted in inhibition of cell growth and induction of apoptosis of osteosarcoma 143B cells.

Keywords: geldanamycin, osteosarcoma, heat shock proteins

Determinants of Drug and Microenvironment Response in Acute Lymphoblastic Leukaemia

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Introduction: Anthracyclines and glucocorticoids (GCs) are used in the treatment of acute lymphoblastic leukaemia (ALL) as they induce apoptosis in lymphoid cells. The GCs induced apoptosis is mediated by the glucocorticoid receptor (GR), whereas anthracyclines activate the tumour suppressor p53.
Objective: The underlying mechanisms of ALL apoptosis and effect of bone marrow microenvironment remain poorly defined. In order to identify determinants of drug sensitivity we performed an integrated analysis of gene expression profiles.
Materials & Methods: Microarray analysis from GC sensitive and resistant ALL cell lines and patients was performed, including new and published data.
Results: We identified 358 differentially regulated genes that we classified into 15 kinetic profiles by applying time-series clustering analysis in the sensitive ALL. We found that activator protein 1 (AP-1), Ets related gene (Erg) and GR pathways were differentially regulated in sensitive and resistant ALL treated with GCs. Erg protein levels were substantially higher in resistant cells and c-Jun was significantly induced by GCs in sensitive cells. c-Jun was recruited to the AP-1 site on the Bim promoter, whereas a transient Erg occupancy on the GR promoter was detected. Inhibition of Erg and activation of GR lead to increased apoptosis of ALL cells. Etoposide and GC combined treatment altered the apoptotic pathways when compared to individual treatments. Bone marrow microenvironment changed expression profiles of genes that control inflammation and increased ALL chemoresistance.
Conclusion: These novel findings advance our understanding of drug sensitivity, link host-tumour interactions to chemoresistance and inflammation, and can be used to improve ALL therapies.

Keywords: glucocorticoid receptor, gene expression, ALL

CGP52411, an Inhibitor of the EGFR Signalling Pathway, Enhances the Chemosensitivity of Laryngeal Squamous Carcinoma Cells to Cisplatin

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Introduction: Carcinogenesis of head and neck results from various dysregulations of cellular proliferation, differentiation, and cell death. Aberrant receptor tyrosine kinase signaling, due to overexpression or activating mutation of receptors and/or their ligands, frequently underlies diverse faces of tumor pathobiology and thus provides an attractive target for cancer therapy.
Objective: We investigated apoptotic effects of Cisplatin combined with RTK inhibitor 5,6-bis (phenylamino)-1H-isoindole-1,3 (2H)-dione (CGP52411, DAPH) on human laryngeal epidermoid carcinoma HEp-2 cell line.
Materials & Methods: HEp-2 cells were treated with CGP52411 alone or in combination with Cisplatin. Cell viability was evaluated using MTT assay. Antibody array analyses were employed to examine alterations of 43 different apoptotic proteins in response to different exogenous stimuli. Next, changes in several apoptosis-related molecules (*CASP3*, *CASP8*, *Bcl-XL* and *Survivin*) were examined using qPCR method.
Results: In HEp-2 human laryngeal epidermoid carcinoma cells, we observed that application of CGP52411 was able to suppress cancer cell proliferation. CGP52411, Cisplatin and combination of both chemicals inhibited the growth of HEp-2 cells in dose-dependent manners (LD_{50} values were 20 µmol/L, 15 µmol/L and 10 µmol/L Cisp + 1 µmol/L CGP, respectively, at 24h). Significant up-regulation of CASP8 mRNA was observed when cells are treated with CGP52411 alone or together with Cisplatin ($P<0.05$). Also we found that expression of CASP3, CASP8, BID, BIM, Fas, Hsp27, Hsp70, HTRA, sTNF-R2, TNF-α, TRAIL1, 2, 3, 4 proteins were partially increased ($P>0.05$).
Conclusion: Collectively, these results suggest that CGP52411 when used together, at clinically achievable concentrations, reduced the LD_{50} values of Cisplatin.

Keywords: CGP52411, Cisplatin, HEp-2 cell line

Anticancer Effects of Prostaglandin J_2 on Highly Metastatic Osteosarcoma 143B Cell Line

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Introduction: Osteosarcoma is one of the most malignant tumors of childhood and adolescence characterized by a high degree of metastatic potential and resistance to chemotherapy. Recent studies demonstrated that prostaglandin J_2 (PGJ_2) could act as potent anticancer and anti-inflammatory compound. However, detailed molecular mechanisms underlying these effects remain to be elucidated.
Objective: The main goal of our project was to study the mechanism of the anticancer and antiproliferative effects of PGJ_2 on human osteosarcoma 143B cell line.
Materials & Methods: Inhibtion of cell growth was assessed by MTT assay. Induction of apoptosis, cell cycle arrest, reactive oxygen (ROS) and nitrogen (RNS) species production were determined by flow cytometry analyses, and the level of iNOS protein by Western-blotting.
Results: The antiproliferative effect of PGJ_2 was concentration dependent with an EC50 value of 10.8±1.6 μM. We observed induction of apoptosis and cell cycle arrest after 24 hr of treatment with PGJ_2. Moreover, PGJ_2 significantly decreased LPS-induced production of ROS, RNS and the level of iNOS in 143B cell line.
Conclusion: PGJ_2 displayed antiproliferative and proapoptotic effects in highly metastatic osteosarcoma 143B cell line. On the other hand, suppression of oxidative stress may suggest its anti-inflammatory properties.

Keywords: PGJ_2, osteosarcoma, oxidative stress

The Effects of Receptor Tyrosine Kinase and JAK/STAT Pathway Inhibitors in Colon Cancer Cell Lines

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Introduction: The Epithelial Growth Factor Receptor (EGFR) is frequently overexpressed in colon cancer. The EGFR-associated JAK-STAT signaling pathway plays an important role in carcinogenesis and cancer progression. Gefitinib (Gef) is an inhibitor of tyrosine kinase that targets the epidermal growth factor receptor (EGFR). Cucurbitacin B (CuB) is a potent and selective inhibitor of JAK-STAT signaling pathway.
Objective: In the present study, we aimed to investigate apoptotic and anti-proliferative effects of CuB alone and in combination with Gef treatment on HT-29 and HCT-116 cells. We examined Bcl-2, Bax, Bak, Bad, cycD1and p27kip1 mRNA and protein expression levels after Gef and CuB treatment.
Materials & Methods: The effects of two agents on cell viability, DNA synthesis rate, cytotoxicity, DNA fragmentation, Relative mRNA and protein expression levels were analyzed using XTT, BrdU-ELISA, LDH release assays, Real-time PCR and western blotting method.
Results: Both Gef and CuB treatment resulted in a decrease in a cell viability and the apoptotic effects of CuB were increased in the presence of Gef. mRNA expression levels of Bcl-2 and cycD1 genes were found to be downregulated in both cell lines. Bax and Bad genes were found to be upregulated in HT-29 cells after Gef and CuB treatment. In HCT-116 cells, Bax and bak mRNA levels significantly increased after Gef and CuB treatment alone and their combinations. These results were compatible with the proteins expression levels.
Conclusion: These data suggest that CuB and Gef+CuB combination could be useful as a potential chemotherapeutic agents in the management for colon cancer.

Keywords: apoptosis, cucurbitacin B, gefitinib, HCT-116 cell line, HT-29 cell line

Complement Inhibition by Depletion with Humanized Cobra Venom Factor (CVF): An Experimental Immunotherapy in Diseases with Complement Pathogenesis

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Introduction: Cobra Venom Factor (CVF) is the anti-complementary protein in cobra venom. Whereas it does not exert direct toxicity, it exhaustively activates complement by forming a stable C3- and C5-cleaving enzyme in serum, called the C3/C5 convertase, leading to complement depletion. CVF and mammalian C3 share both extensive structural and functional homology (such as forming a C3/C5 convertase). In contrast to CVF, the convertase formed with C3b is rather short-lived, consistent with its biological function of localized complement activation on a target cell surface.
Materials & Methods: We have created hybrid proteins of human C3 with CVF by exchanging functionally important regions in human C3 with the corresponding regions from CVF. These human C3 derivatives (called humanized CVF) were tested for forming a stable C3, and for their therapeutic activity in preclinical models of disease with complement pathogenesis.
Results: The hybrid proteins are human C3 derivatives with the CVF-specific function of forming a stable convertase in human serum, causing complement inhibition by consumption. Fortuitously, the convertase formed with humanized CVF does only cleave C3, thereby avoiding generation of the strongly pro-inflammatory C5a anaphylatoxin. We will present results from several preclinical models of disease where complement depletion with humanized CVF is a potent therapeutic approach, including myocardial infarction reperfusion injury, age-related macular degeneration, myasthenia gravis, and others.
Conclusion: Humanized CVF is a novel experimental therapeutic agent for complement depletion in diseases with complement pathogenesis. It may also be useful for suppressing the chronic inflammatory processes in cancers and other diseases.

Keywords: complement, complement depletion, cobra venom factor, humanized cobra venom factor

The Comparative Study of Natural Killer Cell (NK cell) Count in Gynecologic Cancer Patients between Treatment Responders and Non-responders

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Introduction: Natural killer cells (NK cells) are lymphocytes that are programmed to recognize tissues altered by malignant transformation and can be used as prognostic factor in some cancer treatment. This study wanted to view the prognostic value of NK cell levels for predicting the treatment outcomes.
Objective: To compare NK cell levels between the treatment responders and non-responders in gynecologic cancer patients.
Materials & Methods: The serum NK cells were assessed prior to the first course of chemotherapy from gynecologic cancer patients in Siriraj Hospital. The analytical cohort study was conducted between March 2011 and March 2012.
Results: 122 patients were evaluated. Median of NK cell count in non-responder group was 282 cells/μL (range 66-1,885 cells/ μL), compared to 313 cells/ μL (range 95-1,255 cells/ μL) in responder group (p = 0.276). Only 93 patients had available follow-up data, in the mean follow-up time of 7.3 months. Mean NK cell was higher in the patients who alive without disease compared with patients who alive or death with disease (400±240; 326±342; 171±95) respectively (p = 0.001).
Conclusion: Pre-treatment NK cell levels failed to predict the response of chemotherapy. However, the NK cell levels were significantly higher in the patients who alive without disease in the follow-up period compared to the others who alive or death with disease. The further well-design larger study may conduct to evaluate a potential role of NK cell in gynecologic cancer in Thai women.

Keywords: NK cell, ovarian cancer, treatment response

The Prognostic Significance of Matrix Metalloproteinase 11 Expression in Prostatic Adenocarcinoma

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Introduction: Prostate cancer is the most common cancer in elderly men worldwide. Matrix metalloproteinase 11 (MMP11) is the member of matrix degrading proteinases that expressed in fibroblast-like cells of various cancers. It seems to play an important factor in cancer progression and tumorigenesis.
Objective: The aim of the present study was to determine the prognostic value of MMP11 in patients with prostatic adenocarcinoma.
Materials & Methods: Immunohistochemical analysis of MMP11 was carried out in paraffin embedded tissue sections of 113 Thai patients with prostatic adenocarcinoma. Prognostic value of MMP11 and other factors was analyzed using Cox proportional hazard model. Overall probabilities of survival were evaluated using Kaplan-Meier methods.
Results: Immunoreactivity of MMP11 displayed highly with the stromal cells. There was no significant correlation with age, bone metastasis, Gleason's score, stage of cancer, and survival time, but with high level of prostate-specific antigen (PSA) ($p<0.05$). Multivariate analyses showed that MMP11 expression and some clinicopathologic factors (age, PSA levels, and bone metastasis) were no significant associated with time-to-death. However, the Gleason's score and stage of cancer were associated with patients' survival (hazard ratio, 1.74; 95% CI 1.06-2.84; $p=0.027$, hazard ratio, 1.92; 95% CI 1.37-2.68; $p<0.001$, respectively).
Conclusion: MMP11 expression was not a significant prognostic factor in patients with prostatic adenocarcinoma. The Gleason's score and cancer stage were associated with patients' survival.

Keywords: prostate cancer, matrix metalloproteinase (MMP) 11, prognosis

Genetic Variation of *LGALS3* Encoding Galectin-3 Associated with Chemosensitive Cholangiocarcinoma

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Introduction: Cholangiocarcinoma (CCA) – a malignancy of bile duct epithelia, resulting in a high mortality rate and poor prognosis – is the most common cancer in the Northeastern Thailand. Failure to surgery and poor response to chemotherapy usually occurs in the CCA patients. Galectin-3 (Gal-3) expression in CCA cell lines generated from the CCA patients has been found to be associated with response to chemotherapeutic drug. The CCA cell lines with low Gal-3 expression can more easily be induced to apoptosis by anti-cancer drugs than those with high Gal-3 expression. Regulation of Gal-3 expression is a complex process and its mechanisms are still unclear. We propose that one mechanism regulating Gal-3 expression is galectin-3 gene (*LGALS3*) variation and its detection may be useful for more effective treatment of CCA.
Objective: Genetic variations of galectin-3 gene (*LGALS3*) were examined in CCA cell lines expressing different Gal-3 to understand the regulation of its variable expression.
Materials & Methods: Gal-3 mRNA and protein expressions were determined in 11 CCA cell lines by RT-PCR and immunoblotting. All exons of *LGALS3* and its promoter region were amplified from DNA samples prepared from 11 CCA cell lines and leukocytes of normal individuals by PCR. Genetic variations in the amplified fragments were scanned by high resolution melting (HRM) technique and confirmed by DNA sequencing.
Results: Differential expression of Gal-3 in 11 CCA cell lines was observed. KKU-M055, KKU-M139, and KKU-W040 showed low Gal-3 expression. KKU-M055, which had the lowest Gal-3 mRNA and protein expression, contained a C insertion at the promoter of *LGALS3*. In addition, exon-1 of the gene could hardly be amplified and detected in KKU-M055, indicating its deletion. Furthermore, variations of exon-3 sequence were observed in 11 CCA cell lines without the association with Gal-3 expression.
Conclusion: Genetic variation in the promoter of *LGALS3*, which contains transcriptional-binding sequence, and a noticeable deletion in its exon-1 were observed in a chemosensitive CCA cell line. This is thus a possible mechanism regulating low Gal-3 expression in CCA.

Keywords: cholangiocarcinoma, bile duct cancer, galectin-3, *LGALS3*, genetic variation

The Potential of Serum Periostin as a Prognostic Marker in Cholangiocarcinoma Patients

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Introduction: Having good prognostic marker may improve the treatment in cholangiocarcinoma (CCA) patients. Periostin is a multifunctional protein secreted mainly from stromal fibroblasts within CCA tissues and has been elucidated the strong impact in CCA progression.
Objective: We aimed to evaluate level of serum periostin in CCA patients and investigate the correlation with clinicopathological parameters.
Materials & Methods: Sera of 68 CCA patients obtained were collected and measured serum periostin by enzyme-linked immunosorbent assay. Sera from 50 normal controls, 6 benign liver diseases, and 2 hepatocellular carcinoma were enrolled. The diagnostic performance of serum periostin for distinguishing CCA patients from others was also assessed. The resulting data were searched for prognostic markers by appropriate statistical analysis programs.
Results: Serum periostin in CCA patients were statistically higher than that in healthy controls and patients with benign liver diseases ($P<0.05$). With optimal cut-off value, diagnostic performances for CCA from other conditions were as follows: sensitivity 0.68 (95% CI 0.62-0.75); specificity 0.68 (0.61-0.75); accuracy 0.68 (0.61-0.75); positive predictive value 0.38 (0.31-0.45); and negative predictive value 0.88 (0.83-0.93) with statistical significance ($P<0.001$). Moreover, serum periostin indicated short survival time ($P<0.007$) and was a risk factor with hazard ratio 3.222 with statistical significance.
Conclusion: We demonstrated elevated serum periostin in CCA patients and the diagnostic and prognostic predictive value of serum periostin in these patients. This is a great supportive data for the role of stromal microenvironment in CCA aggressiveness. The serum periostin can be suggested as a poor prognostic marker in CCA patients.

Keywords: periostin, cholangiocarcinoma, fibroblast, prognostic marker

Human Single-chain Variable Antibody Fragment (HuScFv) Neutralizing Tautomerase Activity of Macrophage Migration Inhibitory Factor (MIF)

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Introduction: Macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine, secreted from a variety of cells to regulate both the innate and the adaptive immune responses. Elevated serum MIF levels correlate with severity of inflammatory diseases in humans. Inhibition of MIF or its tautomerase activity ameliorates disease severity by reducing inflammatory responses.
Objective: To generate human single-chain antibody variable fragment (HuScFv) that specifically binds to MIF and inhibits MIF tautomerase activity.
Materials & Methods: Human MIF-specific HuScFv clones were screened from the human antibody phage display library by recombinant human MIF (rMIF) protein according to bio-panning procedure. The phages producing HuScFv were selected and HuScFvs were examined for their binding activities to the rMIF protein by indirect ELISA and Western blot analysis. MIF-specific HuScFv were purified and verified for the binding activity to MIF protein. Finally, inhibitory activity of HuScFv on MIF tautomerase activity was determined.
Results: A HuScFv exhibiting the highest binding signal to the rMIF protein could inhibit the tautomerase activities of both rMIF and native MIF prepared from human macrophage (U937) cells in dose-dependent manner. Mimotope and molecular docking analyses demonstrated that the HuScFv interacted with Lys32 and Ile64 within the MIF tautomerase active site.
Conclusion: In this study HuScFv specific to human MIF was generated and this HuScFv could inhibit MIF tautomerase activity because it can bind to MIF tautomerase active site. This is the first report of MIF-specific HuScFv containing tautomerase-inhibitory activity, which is potential to be further developed as anti-inflammatory biomolecules.

Keywords: macrophage migration inhibitory factor, tautomerase activity, human single-chain antibody (HuScFv), inflammation, inflammatory disease

Investigation of Lipidomics from Plasma of Liver Cancer Patients

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Introduction: Major types of liver cancer are hepatocellular carcinoma and cholangiocarcinoma. Sometimes it is difficult to identify tumor type without invasive procedure. Nowadays, serum markers for liver cancer are not appropriate for definite diagnosis. Lipidomics is a new approach for investigation of new biomarkers for cancer, in addition to conventional approaches.
Objectives: In this study, MALDI-TOF was used to investigate distingue plasma lipid peak patterns from cholangiocarcinoma, hepatocellular carcinoma, and biliary stricture patients compared to healthy controls.
Materials & Methods: Lipids from plasma of all patients and 3 healthy controls were extracted by chloroform-methanol. Lipid concentrations were determined by phospho-vanilin reaction. Plasma lipidomics were analyzed by MALDI-TOF with positive ion mode, which 2,5-dihydroxybenzylamine (DHBA):trifluroacetic acid (TFA) 1:4 was used as matrix. The peak results of samples were analyzed by ClinProTools® software version 2.0.
Results: The analysis presented the different lipid peak patterns among each liver disease sample and healthy controls.
Conclusion: Crystallization and dispersion of DHB matrix in lipid sample could affect the reproducibility of the analysis; hence, increase application of each sample could reduce inaccuracy. Lipidomic peak patterns can distingue each liver disease patients from healthy controls. This should indicate the application of lipidomic to diagnose the malignancy of liver. Further study should be the investigation of consistency among higher size of patients for identification of specific abnormal lipids.

Keywords: lipidomics, liver cancer, MALDI-TOF

Olive Leaf Polyphenols Protect Cultured Rat Cardiomyocyte against HNE-induced Damage and Toxicity

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Introduction: 4-Hydroxynonenal (HNE), a by-product of lipid peroxidation, induces apoptosis and degeneration. The phenolic extracts of olive (*Olea europaea* L.) leaves with antioxidant, hypolipidemic, antihyperglycemic and antiproliferative activities has been shown to protect insulin-secreting cells against oxidant-induced injury and inflammation.
Objective: We compared the effects of an oleuropein reach (OLE-1) and a hydroxytyrosol rich (OLE-2) olive leaf extracts with the effects of standard compounds (quercetin, hydroxythyrosole and oleuropein) on HNE-induced toxicity in rat cardiomyocye (H9C2) cell cultures.
Materials & Methods: Cell viability was detected by MTT assay; reactive oxygen species (ROS) levels were assessed using DCFH2-DA; and mitochondrial membrane potential ($\Delta\Psi(m)$) was determined using JC-1 kit. Hsp27, cl-caspase 3, cl-PARP and SAPK/JNK were measured by Western blot. Cells were preincubated with increasing concentrations of each extracts or standards for 24 h and then cultured with HNE.
Results: HNE inhibited the viability (LD_{50}: 25μM) and $\Delta\Psi(m)$, while it markedly increased ROS, apoptosis and oxidative stress-related transcription factors. Both extracts reduced HNE-toxicity, improved viability, attenuated ROS generation and protected $\Delta\Psi(m)$ (0.1-10 μg/ml, $p<0.05$). The effects of extracts on $\Delta\Psi(m)$ was more than the individual effects of the standards ($p<0.05$). SAPK/JNK and Hsp27-induced increase in the presence of HNE was inhibited especially by quercetin (0.1-10 μg/ml, $p<0.05$) and other polyphenols. Pretreatment with oleuropein or OLE-1 (0.1-10 μg/ml, $p<0.05$) induced down-regulation of cl-caspase 3 and cl-PARP in cells under conditions of HNE-induced cellular stress compared to untreated cells.
Conclusion: Olive leaf polyphenols protect cardiomyocytes against HNE-induced toxicity (supported by KOSGEB- No: 2011-0850, Gazi University-01/2012-70, COST-BM1203).

Keywords: 4-hydroxynonenal, oxidative stress, apoptosis, H9C2-cardiomyocytes, olive leaf extracts

Autophagy Induction by Cucurbitacin B in Breast Cancer Cells

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Introduction: Cucurbitacin B, a tetracyclic triterpenoid, is found in the cucurbitaceous plants. It has been shown to possess anticancer and anti-inflammatory activities. In this report, the induction of autophagy by cucurbitacin B isolated from Thai medicinal plant *Trichosanthes cucumerina* L. was tested.
Objective: This study was aimed to investigate autophagic responses of breast cancer cells upon cucurbitacin B treatment.
Materials & Methods: Four breast cancer cells; T47D, MCF-7, MDA-MB 231, SKBR-3 and endothelial breast cell line; HBL-100 were used to observe efficacy of cucurbitacin B on autophagy. Cells were treated with various concentration of cucurbitacin B for the indicated time points then were stained with acridine orange. The staining cells were analyzed using FACS flow cytometer. GFP-LC3 plasmid was transiently transfected into the cells followed by cucurbitacin B treatment and viewed under fluorescence microscope in order to monitor autophagosome formation. The autophagic response was also proved by Western blot analysis. Cells were treated with cucurbitacin B for 24 hours then the proteins were probed using antibodies against either autophagy or apoptosis involving proteins, LC3 and PARP.
Results: The results demonstrated that cucurbitacin B increased percentage of AO positive cells in a time- and dose-dependent manner. Cells treated with cucurbitacin B showed a dot-like pattern of LC3 confirming formation of autophagosomes while untreated cells showed a diffuse distribution of LC3. Western blot results indicated changing in both autophagy (LC3) and apoptosis (PARP) protein patterns.
Conclusion: Our data suggest that cucurbitacin B can induce autophagy as well as apoptosis of breast cancer cells.

Keywords: autophagy, cucurbitacin B, breast cancer

Tumor Reducing and Anti-angiogenic Effects of *Acanthus ebracteatus* Vahl in Nude Mice Implanted with Cervical Cancer

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Introduction: *Acanthus ebracteatus* Vahl. is a medicinal plant, traditionally used against various types of cancer.
Objective: The aim of this study was to examine the effects of the aqueous crude extract of *Acanthus ebracteatus* Vahl (AE) on tumor growth and angiogenesis by utilizing a mice model of HPV-associated cervical cancer.
Materials & Methods: The growth-inhibitory effect of AE was obtained in various cells: CaSki, HeLa, HepG2, and HDFs by MTT assay. To conduct studies *in vivo*, a cervical cancer cell lines (CaSki 1×10^7 cells) was injected subcutaneously at the middle dorsum of each animal (HPV group). One week after injection, mice were fed orally with AE 300 or 3,000 mg/kg BW/day for 14, 28 days. Tumor microvasculature and capillary vascularity were investigated using laser scanning confocal microscopy. The immunostaining of tissue vascular endothelial growth factor (VEGF) and p53 were performed.
Results: The dose-dependent effect of AE on cell growth inhibition was observed. After 48-hr incubation, the IC_{50} of AE in CaSki was discovered to be significantly different from HDFs ($P<0.05$). A large number of microvascular network was observed around the tumor area in all HPV groups and tumor capillary vascularity was significantly increased compared with control group ($P<0.001$). High-dose treatment of AE significantly attenuated the increase VEGF expression, tumor angiogenesis, and tumor growth either both treatment period ($P<0.001$). AE treatment could restore p53 expression in HPV group in a dose-dependent manner ($P<0.001$).
Conclusion: Our novel findings demonstrated that AE could inhibit cervical cancer growth, p53, VEGF expression, and angiogenesis in a CaSki-cell transplant model in mice.

Keywords: *Acanthus ebracteatus* Vahl, tumor angiogenesis, VEGF, CaSki cell-implanted nude mice, capillary vascularity, laser scanning confocal microscopy

Antiproliferative Activity of Quercetin and Its Modified Analog 3,7 -dimethoxy -5,3',4'-trihydroxyflavone

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Introduction: Quercetin is an important flavonoid found in various fruits and vegetables. Many studies have shown that quercetin possesses potential anticancer activity promising cancer chemopreventive compound. However, some limited bioavailability of quercetin present a problem for its administration. Therefore, chemical modification of the quercetin structure may provide an efficacious activity against cancer.
Objective: The aim of this study was to investigate antiproliferative activity of quercetin (3, 3', 4', 5, 7-pentahydroxyflavone) and its structurally modified analog 3,7-dimethoxy-5,3',4'-trihydroxyflavone in human colon cancer and human mouth carcinoma cell lines.
Materials & Methods: Human colon cancer Caco-2 cells and human mouth carcinoma CLS-354 cells were seeded into a 96-well plate at a density of 6×10^3 cells/well. Cells were allowed to grow to approximately 80% confluency for 48 h. Then the cells were treated with quercetin or its analog (0–40 μg/ml) for 24 h. The cell viability was determined by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.
Results: Quercetin and its analog significantly inhibited cancer cell growth in a dose dependent manner. The analog exhibited cytotoxicity against cancer cells better than that quercetin. The half maximal inhibitory concentration (IC_{50}) of the analog were 32 μg/ml (97 μM) and 40 μg/ml (121 μM) in Caco-2 cells and CLS-354 cells, respectively, while the IC_{50} value of quercetin was > 40 μg/ml in both cell lines.
Conclusion: It is indicated that substitution of methoxy moieties in the quercetin structure led to acquisition of greater antiproliferative activity. Thus, chemical modification of the flavonoid structure could provide an efficacious anticancer agent.

Keywords: quercetin, quercetin analog, anticancer activity

Cytotoxic Effect of Andrographolide Isolated from *Andrographis paniculata* on Human Epithelial Cancers

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Introduction: Andrographolide is a major biological active compound found in *Andrographis paniculata*, a medicinal plant widely used in many Asian countries. Andrographolide has been known to possess a broad range of biological properties such as anti-inflammatory, anticancer, and antiviral properties. For anticancer property, andrographolide has been shown to induce cell death by induction of apoptosis in various human cancer cells. However, there has been no report on the cytotoxic effect of andrographolide in two human epithelial cancer Caco-2 and CLS-354 cells.
Objective: The purpose of this study was to investigate the cytotoxic effect of andrographolide on human colon cancer Caco-2 and human mouth carcinoma CLS-354 cell lines.
Materials & Methods: Cells were seeded at a density of 6×10^3 cells/well in a 96-well plate and allowed to grow to approximately 80% confluency for 48 h. After incubation, cells were then treated with andrographolide or standard drug cisplatin for 24 h. Cytotoxicity was investigated by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.
Results: Andrographolide significantly inhibited cancer cell growth in a dose dependent manner with the half maximal inhibitory concentration (IC_{50}) of 71 μM in Caco-2 cells and 20 μM in CLS-354 cells. Cytotoxic activity obtained from andrographolide was comparable to the activity of the commonly used anticancer drug cisplatin with IC_{50} values of 67 μM and 17 μM in Caco-2 and CLS-354 cells, respectively.
Conclusion: Andrographolide could be a promising anti-proliferative agent in cancer therapeutics via its potent inhibitory effect. Mechanisms of action of andrographolide in epithelial cancers will be further elucidated.

Keywords: andrographolide, *Andrographis paniculata*, cytotoxicity

Antimigration and Antiinvasion Effects of *Phyllanthus emblica* Extract on Human Fibrosarcoma Cells

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Introduction: *Phyllanthus emblica* (PE) fruit has been used in traditional medicine due to its wide range of biological activities. Several pharmacological actions were mentioned such as analgesic, antibacterial, anti-inflammatory, antipyretic, antioxidant, antimutagenic, including anticancer actions.
Objective: To evaluate the antimetastatic potential of PE extract on human fibrosarcoma cells.
Materials & Methods: Antimigration of fibrosarcoma cells was assessed by using 48-well chemotaxis chamber and a polycarbonate membrane of 8 μm pore size coated with collagen IV. Fibrosarcoma cells suspension containing PE extract at various concentrations were seeded on the upper compartments and allowed to migrate toward the chemoattractant in the lower compartments. After 4 hr-incubation, the migrated cancer cells adhering to the lower surface of the filter were fixed, stained and counted in five random fields under a light microscope. Percentage of inhibition at 50% of control was used to compare with the migration at each dose of PE extract. Inhibition of the invasion was determined by the same method as in migration assay except that polycarbonate membrane was precoated with Matrigel®.
Results: PE extract could suppress the migration and invasion of fibrosarcoma cells with dose-dependent fashion with IC_{50} values at 0.64 ± 0.07 and 0.75 ± 0.06 mg/ml, respectively.
Conclusion: Our data showed that PE extract could significantly suppress the migration and invasion of human fibrosarcoma cells. It could imply that PE extract has a high potential for the prevention and treatment of metastasis of human fibrosarcoma in addition to the conventional treatment. Further investigations to determine the molecular mechanism are worthwhile.

Keywords: *Phyllanthus emblica*, migration, invasion, human fibrosarcoma cells

Carotenoid Intake and Supplementation in Cancer – Pro and Con

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Introduction: After beta-carotene (BC) failed in clinical cancer trials, there is evidence that BC might have harmful effects at high dosages.
Objective: Negative side effects might be mediated by BC breakdown products (BCBP). Within BCBP there is a multitude of aldehydic and epoxidic coumpounds, which exert similar effects compared with hydroxynonenal. BC is cleaved non-enzymatically by liberated oxidants of stimulated neutrophils (SN). Supplementation of BC seems to be important in various diseases including cancer. It is necessary to think on safe conditions for supplementation.
Materials & Methods: BC degradation was investigated under various conditions. BC was rapidly degraded by SN, too. Hepatocytic mitochondrial respiration and genotoxicity were evaluated in presence of BCBP. In parallel experiments antioxidants were used to avoid negative side effects.
Results: BCBP modify the activities of enzymes and transport proteins such as Na-K-ATPase, NADPH oxidase, and adenine nucleotide translocator. At nM concentrations BCBP exert genotoxic effects. BCBP impair mitochondrial functions. During incubation of mitochondria with BCBP GSH and protein SH decreased, lipid peroxidation increased. SN mediated BCBP formation was reduced by antioxidants. The inhibition of ADP-stimulated respiration was also mitigated by antioxidants. Even genotoxic effects could be avoided if antioxidants were present. Best protective effects were exerted by vitamin E.
Conclusion: The data indicate a basic pro-oxidative mechanism of high concentrated BC at heavy oxidative stress leading to rapid formation of BCBP. Antioxidants mitigate potential toxic effects. Endogenous conditions and dosage decide on Pro and Con of carotenoid intake and supplementation in cancer.

Keywords: carotenoids, cancer, beta-carotene breakdown, antioxidants, neutrophils

Ginsenoside Compound K Inhibits Angiogenesis *via* Regulation of Sphingosine Kinase-1 in Human Umbilical Vein Endothelial Cells

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Introduction: Ginsenosides found in the plant genus Panax ginseng (Araliaceae) display diverse biological activities, including protection against the development of cancer, inflammation, allergies, and diabetes. Ginsenosides Rg3 and compound K (CK) reportedly inhibit vascular endothelial growth factor-induced cell proliferation, tube formation, and chemoinvasion in human umbilical vein endothelial cells (HUVECs). By contrast, ginsenosides Rg1 and Re stimulate cell proliferation, tube formation, and migration in these cells.
Objective: Sphingosine kinase 1 (SPHK1) and its related product, sphingosine 1-phosphate (S1P), are also involved in cell proliferation, migration, and protection of apoptosis; therefore, we sought to investigate whether ginsenosides are able to regulate SPHK1.
Materials & Methods: For this purpose, we developed an inhibitory assay of SPHK1 activity and an analytical method for detection of S1P and other sphingolipid metabolites in HUVECs.
Results: Ginsenoside CK, which is an intestinal metabolite isolated from ginseng protopanaxadiolsaponins, inhibited SPHK1 activity, S1P production, and HUVEC proliferation, whereas expression of the pro-apoptotic sphingolipids, sphingosine and ceramide, was increased in response to CK. The major subspecies of the increased ceramide was C24:0-ceramide. CK also disrupted the sphingolipid rheostat, which ultimately influences cell fate, and dose-dependently inhibited HUVEC migration by reducing expression of metalloproteinases (MMPs).
Conclusion: In conclusion, ginsenoside CK acts as a unique HUVEC migration inhibitor by regulating MMP expression, as well as activity of SPHK1 and its related sphingolipid metabolites.

Keywords: Araliaceae (Panax ginseng), ginsenoside, compound K, sphingosine kinase, angiogenesis, human umbilical vein endothelial cells

Anti-inflammatory Activity of Indigenous Thai Vegetables in Intestinal Like Caco-2 Cell

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Introduction: Chronic diseases are global public health problem including Thailand. Overproduction of inflammatory mediators and free radicals is risk factor of several chronic diseases. Phytochemicals in dietary plants have been shown to alleviate clinical pathology by decreasing inflammatory mediators and free radicals *in vitro* and *in vivo*.
Objective: To assess anti-inflammatory activity from indigenous vegetables [*Albizia lebbeck* (Ta-kuek), *Melientha suavis* (Pak-Wan-pa), *Millettia brandisiana* (Kra-phi-chan)] in inflamed intestinal like Caco-2 cells.
Materials & Methods: Ethanol extract of freeze dry at 2.5-10 mg/ml was incubated with Caco-2 cells for 4 h prior to activate with H_2O_2 and IL-1β for another 24 h. The anti-inflammatory activity was assessed by measuring IL-8 and MCP-1 levels in cell treated culture media by ELISA and antioxidant capacity was assessed by measuring reactive oxygen species (ROS) in the cell lysate by fluorometer.
Results: The extracts from three indigenous vegetables significantly inhibit IL-8, MCP-1 and ROS producing by inflamed Caco-2 cells in a dose-dependent manner.
Conclusion: These data demonstrated that indigenous Thai vegetables have high potential to alleviate clinical outcomes of chronic diseases associated inflammation by attenuation of inflammatory level and free radical level. However, *in vivo* study need be conducted to confirm these biological functions.

Keywords: indigenous Thai vegetables, anti-inflammatory activity, antioxidant activity

Cannabinoid Receptor-independent Anti-proliferative Effect of Docosahexaenoyl Ethanolamide in Head and Neck Squamous Cell Carcinoma Cells

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Introduction: It has been suggested that endocannabinoids, anandamide (AEA) and 2-arachidonoyl glycerol (2-AG), might be the promising anti-cancer agents in clinical fields of cancer treatment. We previously observed that AEA inhibited effectively cell proliferation of head and neck squamous cell carcinoma (HNSCC) cell lines by their receptors-independent action.
Objective: In this study, we tried to check the anti-cancer effects of omega-3 ethanolamides in three HNSCC cell lines (SNU-1041, 1066 and 1076). Docosahexaenoyl ethanolamide (DHEA) and Eicosapentaenoyl ethanolamide (EPEA) are polyunsaturated fatty acid-based ethanolamides like AEA and are known to be increased in human body by dietary supplement with omega-3 fatty acids (DHA and EPA).
Materials & Methods and Results: Similarly to AEA, DHEA inhibited more effectively cell proliferation of HNSCC cells than DHA but EPEA did not. The anti-cancer effect of DHEA seemed to be mediated by cannabinoid receptor-independent action since the antagonist of CB1 and VR1 (AM251, cay10448 and capsazepine) did not reverse DHEA-inhibited cell proliferation (no CB2 was detected in our cell model). Instead, we observed the increase of reactive oxygen species (ROS) and 8-isoprostane production by DHEA. Finally, we identified that treatment with antioxidants (NAC and ebselen) reversed DHEA-inhibited cell proliferation partially.
Conclusion: From these findings, ROS provoked by DHEA seems to play a partial role in anti-cancer effect of DHEA in HNSCC cells. Also, our observations suggest the possibility that DHEA induced by dietary supplement of DHA might mediate the anti-cancer effect of DHA in some cancers such as HNSCC.

Keywords: endocannabinoid, DHEA, ROS, anticancer drug

Cytotoxicity and Mechanism of Action of *Strobilanthes crispus* Extracts on Selected Cancer Cell Lines

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Introduction: *Strobilanthes crispus* has been used as anti-diabetic, diuretic and laxative in traditional folk medicine. Publications on the anti-tumor effects of the plant suggest its potential as an alternative medicine in treating cancers.
Objective: The study aim to determine the cytotoxic effect of various *S. crispus* extracts on selected cancer cell lines. Apoptotic effect of the extracts through caspases activation was also elucidated.
Materials & Methods: *S. crispus* leaves and stems extracts were prepared using hexane, chloroform, ethyl acetate, methanol and water (designated as LH, LC, LEA, LM, LW, SH, SC, SEA, SM and SW). Cytotoxicity of the extracts on nasopharyngeal (CNE1), breast (MDA-MB-231), cervical (HeLa) and liver (HepG2) cancer cells was tested by MTT assay. Apoptosis and caspases activity of the cells were determined by flow cytometry and caspase detection kits, respectively.
Results: LH, LC, LEA, SH, SC and SEA showed cytotoxicity on CNE1 cells while LC, LEA, SH and SC showed effect on HepG2 cells. MDA-MB-231 cells were responsive to LC and SH while HeLa cells only responded to SH. Increased sub-G apoptotic population upon treatment with LC, LEA, SH and SC in CNE1 and LC, LEA and SC in HepG2 cells were noted. Increased apoptosis were also found in SH-treated MDA-MB-231 and HeLa cells. Caspase 3/7 was activated in LEA-treated HepG2 and SH-treated HeLa cells. There were no changes in caspase 8 and 9 expressions in all the cells.
Conclusion: *S. crispus* extracts showed cytotoxicity on various cancer cells and might serve as a potential anti-cancer agent.

Keywords: *Strobilanthes crispus*, cancer, cytotoxicity, apoptosis, caspase

Apoptotic Effect of Alpha-mangostin on Head and Neck Squamous Carcinoma Cells

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Introduction: Head and neck squamous cell carcinoma (HNSCC) is the sixth most common malignancy and causes morbidity and mortality worldwide. The survival rate of HNSCC patients has not been substantially improved by treatment with current chemotherapeutic agents. Alpha-mangostin is a plant antitumor xanthone isolated from *Garcinia mangostana* Linn. *In vitro* studies of alpha-mangostin demonstrated broad antitumor activity against various human tumor cell lines. However, there has been no trial of alpha-mangostin against HNSCC cell lines.
Objective: The purposes of this study were to measure the cytotoxic effect of alpha-mangostin on HNSCC cell lines, to evaluate the apoptotic aspect of dead cells, and to identify the molecular mechanism involved in apoptosis.
Materials & Methods: The human HNSCC cell lines HN-22, HN-30 and HN-31 were treated with alpha-mangostin. The apoptotic effects of alpha-mangostin on HNSCC cells were determined by observation the morphological changes of cells, immunofluorescence for single-stranded DNA, and DNA fragmentation analysis. The expression of bax, bcl-2, and p53 were detected by RT-PCR and Western blot analysis.
Results: Alpha-mangostin showed excellent apoptotic effects on HNSCC cell lines, which induced the down-regulation of bcl-2, but up-regulation of bax and p53 in HN-22, HN-30 and HN-31.
Conclusion: This study is the first report to demonstrate that alpha-mangostin showed apoptotic induction in a concentration- and time-dependent manner in HNSCC cell lines. Furthermore, the induction of apoptosis by alpha-mangostin seemed to be modulated by bcl-2, bax and p53 levels. From these results, it was suggested that alpha-mangostin might be a potential therapeutic agent for HNSCC.

Keywords: alpha-mangostin, apoptosis, HNSCC

Increase of the Number of Tumor-infiltrating Lymphocytes by 6-gingerol in Experimental Murine Tumors

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Introduction: 6-Gingerol is the major pungent principle of ginger, which is consumed as a spice. Mounting *in vitro* evidence indicates 6-gingerol might produce anti-tumor effects. However, little is known for its effects on anti-tumor immune responses against established tumors.
Objective: This study is to analyze effects of dietary 6-gingerol on anti-tumor immune responses in murine tumor model.
Materials & Methods: Balb/c mice were subcutaneously injected with CT26 colon carcinoma cells and were fed with 6-gingerol-containing water until the end of the experiments. Tumor size, the number and subtypes of tumor infiltrating lymphocytes (TILs) and anti-tumor activities of TILs were analyzed.
Results: 6-Gingerol treatment caused massive infiltration of CD4+, CD8+ and B220+, but reduced the number of CD4+Foxp3+ cells. CD8+ cells in TILs isolated from 6-gingerol mice strongly expressed IFN-gamma, a marker of activation of cytotoxic T lymphocytes (CTL) CD107a and chemokine receptors that are expressed on TH1 cells.
Conclusion: Our results suggest that dietary 6-gingerol can increase the number and activities of TILs to produce anti-tumor immune responses.

Keywords: 6-gingerol, tumor infiltrating lymphocytes, tumor immunotherapy

Anti-inflammatory Activities of Endogenous Nicotinergic Peptides in Experimental Models of Inflammatory Bowel Disease

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Introduction: Treatment of inflammatory bowel disease represents serious medical problem. Nicotine enemas can ameliorate both ulcerative colitis and Crohn's disease but severe side effects prompt a search for non-toxic nicotinergic agents mimicking anti-inflammatory effects of nicotine. A novel paradigm of regulation of inflammation was discovered in studies of secreted mammalian Ly-6/urokinase plasminogen activator receptor-related proteins (SLURPs)-1 and -2 that also act upon nicotinic receptors.
Objective: To evaluate anti-inflammatory effects of SLURP-1 and -2 in inflammatory bowel disease.
Materials & Methods: BALB/c mice with oxazolone- or TNBS-induced colitides were treated intrarectally by enemas containing recombinant SLURP-1 and/or -2. To evaluate direct immunoregulatory effects of SLURPs, we used naïve $CD4^+CD62L^+$ T cells (T-c) from BALB/c mice, and human macrophage-like U937 cells stimulated with LPS.
Results: While SLURP-1 abolished predominantly oxazolone colitis and SLURP-2 ameliorated TNBS colitis, a mixture of SLURP-1 and -2 treated both forms of experimental inflammatory bowel disease with a higher efficacy than each SLURP given alone. In experiments with naïve $CD4^+CD62L^+$ T-c, SLURP-1 decreased expression of IFN and IL-17 mRNAs, whereas SLURP-2 downregulated expression of the IL-17 gene and upregulated that of IL-10. Incubation of LPS-stimulated U937 cells with SLURP-1 or -2 in both cases upregulated IL-10 production. SLURP-1 also downregulated IL-1β secretion, and SLURP-2 reduced IL-6. Consistent with the synergistic therapeutic effects of both SLURP-1 and -2, combining the two peptides amplified their anti-inflammatory effect in both test systems.
Conclusion: SLURP-1 and -2 simulate anti-inflammatory effects of nicotine, and both T-c and macrophages can be targeted by SLURP peptides.

Keywords: SLURP-1 and -2, TNBS-colitis, oxazolone-colitis, T cells, macrophages

The Effect of Thymoquinone and Genistein Treatment on Telomerase Activity and Apoptosis in Tyroid Cancer Cell Lines

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Introduction: Anaplastic, that were developed from the stem cells and follicular thyroid cancers are malignant tumours. Thymoquinone, the most important component of the plant Nigella sativa, and Genistein, a component of soy bean have been reported to exhibit antitumorigenic effects.
Objective: A major problems of cancer chemotherapy is resistant of cancer stem cells and the toxic effect on normal cells to current treatments. Therefore, this study was aimed to develop in order to target with high selectivity and activity on tumour cells, which also include cancer stem cells and with minimally toxic effects on normal cells.
Materials & Methods: We investigated the effects of individual and combined dose applications of Thymoquinone and Genistein on telomerase activity, angiogenesis and apoptosis as compared in anaplastic (CAL-62) and follicular (CGTH-W1) thyroid cancer cell lines. Cell viability, mRNA levels of hTERT, PTEN, NF-kB, p21 and VEGF-A genes and active caspase-3 protein level have been measured by MTT assay, Real-time PCR assay and Caspase-3 Sandwich ELISA kit, respectively.
Results: Our results indicate that especially the combined dose application of Thymoquinone and Genistein resulted in statistically high level of apoptosis induction and inhibition of telomerase and angiogenesis in thyroid cancer cell lines. Moreover, thyroid cancer stem cells were found more sensitive to treatment effects of Thymoquinone and Genistein as compared thyroid cancer cell lines which both of cancer stem cell and non-cancer stem cell for the first-time.
Conclusion: These data indicated that the combined use of Thymoquinone and Genistein can be good choice of potential chemotherapeutic agents in the management of thyroid tumours.

Keywords: apoptosis, cancer stem cell, genistein, telomerase, thymoquinone, thyroid cancer

Eryngium foetidum Leaf Extract Inhibit Inflammatory Mediators Secreted by H_2O_2 and IL-1β Induced Caco-2 cells

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Introduction: *Eryngium foetidum* leaf (fEF) is a common vegetable in several Thai recipes. It has long been used as a traditional medicine to treat various diseases. The leaves contain several bioactive compounds which have been demonstrated to possess antioxidant and anti-inflammatory activity; however the underlying mechanism exerting those biological functions has been rarely investigated.
Objective: To evaluate anti-inflammatory activity from fEF ethanol extract in H_2O_2 and IL-1β induced intestinal-like Caco-2 cells.
Materials & Methods: The 11-14 post-confluence Caco-2 cells were incubated with 0.5-2.0 mg/ml ethanol extract for 4 h prior to stimulate with H_2O_2 and IL-1β for another 20 h. The treated cell culture media were collected to determine TNF-α, IL-6, IL-8 and MCP-1 by ELISA. The cell lysate was collected to measure COX-2 by Western blot.
Results: H_2O_2 and IL-1β markedly enhanced TNF-α, IL-6, IL-8, MCP-1 and COX-2 levels in Caco-2 cells. Prior treatment with fEF extract significantly suppressed TNF-α, IL-6, IL-8, MCP-1 and COX-2 production by inflamed Caco-2 cells in a dose dependent manner.
Conclusion: These results suggest that phytochemicals from fEF extract contribute to exhibit anti-inflammatory activities observed in the present study. Thus, *E. foetidum* leaves have potential to reduce clinical outcomes of chronic diseases associated with overproduction of inflammatory cytokines.

Keywords: *E. foetidum*, inflammatory mediators, Caco-2 cells

Differential Proteomic Analysis on the Effects of 2-Methoxy-1,4 -Naphthoquinone (MNQ) towards a Human Metastatic Breast Cancer Cell Line (MDA-MB-231)

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Introduction: We have previously reported the antimetastatic effects of 2-Methoxy-1,4-Naphthoquinone (MNQ), a phytochemical isolated from the plant *Impatiens Balsamina* Linn.
Objective: To investigate the molecular mechanism of action of MNQ in exerting an antimetastatic effect by using the comparative proteomic approach.
Materials & Methods: The whole cell lysates of untreated and 7.5 μM MNQ- treated MDA-MB-231 cells were subjected to two-dimensional gel electrophoresis (2DGE). Protein spots that were differentially expressed by more than 1.5 fold ($p < 0.05$) were identified via matrix-assisted laser desorption ionization tandem time-of-flight mass spectrometry (MALDI-TOF-TOF-MS). Enrichment in Gene Ontology (GO) terms for identified proteins was performed using the GeneCodis3 web-based tools.
Results: Significant modulation of the MDA-MB-231 cell proteome was observed upon treatment with MNQ in which the expressions of 19 proteins were found to be downregulated whereas another eight were upregulated. GeneCodis3 analysis revealed that the proteins affected by MNQ were of diverse functions and profiles. These included cell morphogenesis, protein modification, apoptosis, RNA metabolism, ribosome biogenesis and others. Some of these proteins are also cytoskeleton-related and are calcium-dependent in their activities. Moreover, it is noteworthy that one of the MNQ-downregulated proteins, protein S100A4 is widely implicated in metastatic progression.
Conclusion: Overall, the differential protein expressions induced by MNQ observed in this study may help to uncover its mode of action in exerting an antimetastatic effect. Further works such as the validation of the proteomic dataset and the signaling pathway analysis are currently in progress.

Keywords: 2-methoxy-1,4-naphthoquinone, breast cancer, metastasis, proteomics

Inhibitory Effect of *Spirulina platensis* Extract and Phycocyanin on Epstein-Barr Virus

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Introduction: Epstein-Barr virus (EBV) is a class 1 carcinogen human gamma herpes virus which has been linked to the pathogenesis of human tumors and disorders such as Burkitt's lymphoma and nasopharyngeal carcinoma. *Spirulina platensis*, a cyanobacteria has been consumed as health food. It is rich in phycocyanin which is high in anti-oxidation and anti-inflammatory activities.
Objective: To investigate the effect of *Spirulina platensis* extract and phycocyanin against the release of cell-free EBV DNA and the expression of EBV proteins.
Materials & Methods: The effect of alga extract and phycocyanin was elucidated based on their inhibition efficacy in reducing the number of EBV viral particles being released by Burkitt's lymphoma cell lines, namely B95-8 and P3HR-1. This was assessed using real-time PCR technique. In addition, the effect of alga extract and phycocyanin against the expression of the viral lytic proteins early antigen-restricted (EA-R) and ribonucleotide reductase (RR) was assessed using ELISA method.
Results: *Spirulina platensis* aqueous extract reduced the release of EBV by 50% at approximately 150 μg/mL compared to negative control. At 200 μg/mL, it reduced more than 50% of EA-R and RR expressed in P3HR-1 cells. Relatively, phycocyanin did not show strong antiviral activity against EBV.
Conclusion: *Spirulina platensis* aqueous extract exhibited antiviral activity against the release of cell-free EBV DNA and the expression of EA-R and RR in P3HR-1 cell line. In comparison, phycocyanin did not show a significant antiviral activity against EBV. Therefore, the potential of *Spirulina platensis* as chemoprevention agent against EBV is worth exploring.

Keywords: Epstein-Barr virus, *Spirulina platensis*, phycocyanin

Chrysin Isolated from Thai Propolis Potentiates TRAIL-induced Apoptosis in Cancer Cells through STAT3 Inhibition

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Introduction: TNF-related apoptosis-inducing ligand (TRAIL) selectively kills various types of cancer cells without harmful to normal cells, however, TRAIL resistance has been observed. Propolis (bee glue) is a mixture material collected from various plants by honey bees, it is being attractive for pharmacological research as a rich source of bioactive compounds.
Objective: This study aims to investigate TRAIL sensitization effect of Thai propolis, and to investigate the mechanism of action.
Materials & Methods: Viability of cells in 96-well plate was determined by MTT assay. NF-κB transcriptional activity was measured by reporter assay. Expression levels of apoptosis-related and cell signaling proteins were assessed by immunoblot analysis. Gene expression was determined by quantitative real-time PCR. siRNA transfection was performed using Lipofectamine method.
Results: Thai propolis extract potentiated TRAIL cytotoxicity in a TRAIL-resistant human lung cancer cell line, A549. Chrysin - a major constituent of Thai propolis extract, enhanced TRAIL-induced apoptosis in cancer cells. Chrysin did not inhibit TRAIL-induced NF-κB activation. TRAIL sensitization effect of chrysin was not mediated by oxidative stress. Focus on apoptotic signaling, chrysin selectively decreased Mcl-1 anti-apoptotic protein levels through down-regulation of gene expression. Among the signaling pathways that regulate Mcl-1 gene expression, only constitutive STAT3 tyrosine phosphorylation was suppressed by chrysin. Silencing of Mcl-1 expression by siRNA transfection, or STAT3 inhibitor treatment resulted in TRAIL sensitization similar to chrysin, confirming that inhibition of STAT3/Mcl-1 pathway is the mechanism of chrysin for TRAIL sensitization effect.
Conclusion: In this study, we demonstrated a mechanism of chrysin for TRAIL sensitization by inhibiting STAT3 signaling pathway.

Keywords: STAT3, TRAIL, apoptosis, natural products, cancer

The Influence of Rooibos and Flavonoids on Adrenal Steroidogenesis: UPLC-MS/MS Quantification of Steroid Metabolites in H295R Cells

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Introduction: The steroid hormone output of the adrenal gland is crucial in the maintenance of hormonal homeostasis, with imbalances being associated with numerous clinical conditions which include, amongst others, hypertension, metabolic syndrome and cardiovascular disease. *Aspalathus linearis* (Rooibos), a herbal tea reported to aid stress-related symptoms linked to metabolic diseases, contains a wide spectrum of bioactive phenolic compounds, of which aspalathin is unique.
Objective: To determine the outcome of adrenal steroidogenesis in the presence of Rooibos, aspalathin, nothofagin and rutin.
Materials & Methods: A novel UPLC–MS/MS method was developed for the detection and quantification of twenty-one adrenal steroid metabolites using a single chromatographic separation. Steroid metabolism was assayed in H295R adrenal cells in the absence and presence of Rooibos extract and phenolic compounds under basal and forskolin stimulated conditions, imitating the stress response.
Results: The inhibitory effect of Rooibos on the total steroid output was greater under stimulated conditions, with glucocorticoid biosynthesis being reduced significantly by Rooibos (5.0-fold), aspalathin (1.3-fold), nothofagin (1.7-fold) and rutin (3.0-fold). The inhibitory effect of rutin was observed in the mineralocorticoid, glucocorticoid and androgen pathways under stimulated conditions only, while the inhibitory effect of aspalathin and nothofagin was observed on the latter two pathways only, under both basal and stimulated conditions.
Conclusion: Inhibition of steroid hormone biosynthesis was most apparent under stimulated conditions, with Rooibos and phenolic compounds altering the flux through the steroidogenic pathways, however, the phenolic compounds did not reflect the same effects brought about by Rooibos.

Keywords: Rooibos, adrenal steroidogenesis, glucocorticoids, UPLC-MS/MS

Curcumin Analogs Inhibited Nitric Oxide Secretion in LPS-activated RAW 264.7 Cells

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Introduction: Nitric oxide is an inflammatory mediator which plays an important role in inflammation. High levels of nitric oxide induce severe inflammation leading to inflammatory-associated diseases. Curcumin which is a polyphenol derived from *Curcuma longa* and its analogs are known to have anti-inflammatory activity.
Objective: The aim of this study was to investigate anti-inflammatory activity of curcumin analogs that were 1-(3-nitrophenyl)-7-(2,5-dimethoxyphenyl)-(1*E*,4*E*,6*E*)-1,4,6-heptatrien-3-one (AS-WK013) and 1-(3-hydroxyphenyl)-7-(2,5-dimethoxyphenyl)-(1*E*,4*E*,6*E*)-1,4,6-heptatrien-3-one (AS-WK014) in lipopolysaccharide (LPS)-activated RAW 264.7 cells.
Materials & Methods: RAW 264.7 cells at a density of $3x10^5$ cells/cm^2 were cultured and further treated with various concentrations of AS-WK013 and AS-WK014. After 48 h incubation, cytotoxicity test was performed by MTT assay. For anti-inflammatory assay, cells were treated with both compounds in the presence or absence of LPS (1 μg/ml) for 24 h. Then, culture media was subjected for nitrite measurement (end product of nitric oxide) by Griess assay.
Results: AS-WK013 and AS-WK014 affected cell growth in a dose dependent manner with the half maximal inhibitory concentration (IC_{50}) of 39.2 μM and 13.8 μM, respectively. For their anti-inflammatory activity, the maximum non-toxic concentration was chosen. AS-WK013 (6.84 μM) and AS-WK014 (7.43 μM) revealed significant reduction in nitrite levels in LPS-activated RAW 264.7 cells by 46% and 47% inhibition, respectively ($p<0.05$) compared with 44% inhibition of 5-amino salicylic acid (5-ASA), anti-inflammatory drug.
Conclusion: It is suggested that both compounds exhibited anti-inflammatory activity by reduction of nitric oxide secretion. Thus, the compounds may be used as new anti-inflammatory agents. It will also be important to further elucidate mechanisms of action.

Keywords: anti-inflammation, curcumin analogs, nitric oxide

Eupatorium chinensis var. simplicifolium Root Extract Inhibits the Lipopolysaccharide-induced Inflammatory Response in Raw 264.7 Macrophages by Inhibiting iNOS and COX-2 Expression

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Introduction: Inflammation is a host defense mechanism that is activated in response to harmful substances or pathogens. However, an excessive inflammatory response is a problem in itself. Macrophages secrete inflammatory mediators such as nitric oxide (NO) or cytokines through various pathways such as the nuclear factor kappa B (NF-κB)-activated pathway after recognizing pathogen-like lipopolysaccharide (LPS).
Objective: In this study, the anti-inflammatory effects of *Eupatorium chinensis var. simplicifolium* (EUC) extracts were investigated using LPS-stimulated RAW 264.7 macrophages.
Results: EUC root extract significantly reduced NO production, inducible nitric oxide synthase (iNOS) expression, and cyclooxygenase-2 expression in a concentration-dependent manner. In addition, EUC root extract reduced phosphorylation of mitogen activated protein kinases and protein kinase B, which is upstream of NF-κB. EUC root extract also reduced the degradation of inhibitory kappa B.
Conclusion: These results indicate that EUC root extract exerts anti-inflammatory effects that are mediated by inhibition of iNOS expression and the NF-κB pathway.

Keywords: *Eupatorium chinensis var. simplicifolium*, inflammation, nitric oxide, lipopolysaccharide, NF-κB

Rooibos: A Functional Food in the Management of Metabolic Disorders ?

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Introduction: Rooibos (*Aspalathus linearis*), a South African herbal tea rich in polyphenols, improves lipid profiles and redox status in humans at risk for developing cardiovascular disease (CVD) while also exhibiting hypoglycemic properties.
Objective: To investigate the influence of Rooibos on *in vitro* and *in vivo* glucocorticoid biosynthesis and inactivation, and on cytokine secretion.
Materials & Methods: We investigated glucocorticoid production in H295R cells and cortisol inactivation by 11β-hydroxysteroid dehydrogenase (11βHSD) in CHO-K1 cells in the presence of Rooibos. Plasma steroids levels in rats and humans at risk for CVD were analyzed after Rooibos consumption, by UPLC-MS/MS. IL-6 and IL-10 were investigated in rats by immunohistochemistry.
Results: Rooibos reduced ($P<0.001$) glucocorticoid biosynthesis in H295R cells: cortisol (4.9-fold), corticosterone (5.2-fold). 11βHSD1 was inhibited and the cortisol/cortisone ratio decreased (68%). In rats, Rooibos lowered corticosterone (35%, $P<0.05$), 11-dehydrocorticosterone (18%) and the corticosterone /11-dehydrocorticosterone ratio (20%). In humans, cortisol levels decreased in females (17%), while cortisol/cortisone ratios decreased in both males and females. Rooibos increased testosterone levels in humans and rats, decreasing glucocorticoid/testosterone ratios in humans (28%) and in rats (59%). In rat adrenals, immunohistochemical analysis of cortical tissue showed Rooibos increased IL-10 secretion, while inhibiting IL-6 secretion.
Conclusion: Rooibos inhibited *in vitro* glucocorticoid production under conditions mimicking the stress response, and altered 11βHSD1 activity, favouring glucocorticoid inactivation. The lowered *in vivo* glucocorticoid levels together with the increased anti-inflammatory IL-10 and inhibited pro-inflammatory IL-6 secretion, suggests that Rooibos may serve as a protective agent against metabolic diseases.

Keywords: polyphenols, glucocorticoids, stress, anti-inflammatory cytokines

Poster Presentation Award C-24

Effects of Cucurbitacin B on Breast Cancer Cells: *in vivo*

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Introduction: Cucurbitacin B is one of attractive compound uses for anti-cancer researches. CuB is shown to have anti-cancer properties in several types of cancers including breast cancer. Thus the studying of CuB *in vivo* activity could be the most effective way to prove the potency of this agent for the future breast cancer treatment.
Objective: Cucurbitacin B was tested for their efficacy on growth reduction of tumor in nude mice. Molecular mechanisms of this agent were investigated, focusing on the Wnt-signaling pathway.
Materials & Methods: SKBR-3 cells were subcutaneously injected into the breast of nude mice, which were then intraperitoneally received CuB 1.0 mg/kg every two days after implantation. Tumor size was measured. The tumor volume was then calculated. At the end of experiment, mice were sacrificed and the tumors were weighed. Genes and proteins expressions related in the Wnt-signaling pathway were investigated.
Results: Cucurbitacin B potently inhibited growth of tumors *in vivo*. The dissected tumors in CuB-treated mice significantly had the weight less than those in the control. The mice receiving CuB appeared similar to the control with respect to the external appearance and vitality. Gene and protein expressions in CuB-treated group revealed significantly down-regulation of β-catenin, galactin-3 and cyclinD1.
Conclusion: These results obviously demonstrated that CuB has anti-growth property against xenografts of human breast cancer cells. This compound effectively down-regulates β-catenin, galactin-3 and cyclinD1 in both the mRNA and protein levels. Our finding implies that CuB is an attractive compound for the new therapeutic approach of breast cancer.

Keywords: cucurbitacin B, Wnt signal, β-catenin, galactin-3, cyclinD1

Anti-cancer Properties of *Strobilanthes crispus* Crude Extract and Cytotoxicity of Its Fractions on Liver Cancer (HepG2) Cells

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Introduction: Due to limited success of current anti-cancer therapeutics, the trend now has been shifted towards the search of natural products as a better anti-cancer therapeutic. *Strobilanthes crispus* has been traditionally used as anti-diabetic, diuretic and laxative agents as well as reported to possess anti-viral properties. It has been widely used in cancer management particularly in South Asian region, in which promising outcomes has been shown on various carcinomas.
Objective: This study aimed to determine the cytotoxic and anti-proliferative activities of *S. crispus* crude extracts on liver cancer cells. Studies also aimed to identify anti-cancer bioactive fractions.
Materials & Methods: Ethyl acetate extract prepared from *S. crispus* leaves was tested for cytotoxicity on HepG2 cells using MTT assay. Effect of the extract on cell proliferation was also investigated by determining the cell doubling time. The crude extract was then subjected to column chromatography to fractionate and isolate bioactive components. Anti-cancer property of these fractions was tested through MTT assay.
Results: HepG2 cells treated with ethyl acetate extract showed marked reduction in cell viability with half maximal inhibitory concentration (IC_{50}) of 176.7±15.3 µg/mL. Cells treated with extract showed delayed proliferation with longer cell doubling time as compared to the control. However, the data was not statistically significant. Nineteen fractions were collected from column chromatography. Among the fractions, only fraction 18 (F18) showed cytotoxic effect with IC_{50} of 43.25±1.06 µg/mL.
Conclusion: The results showed that *S. crispus* extract possess anti-liver cancer property, with the bioactive components were found in F18.

Keywords: cytotoxicity, liver cancer, *Strobilanthes crispus*

Inhibitory Effect of PG-platycodin D on the Development of Atopic Dermatitis-like Skin Lesions in ICR Mice

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Introduction: Atopic dermatitis is a inflammatory disease which has complex cause that encompasses immunological responses. Allergic response is associated with mast cells, through the release arachidonic acid, proinflammatory cytokines, and histamine.
Objective: This study shows that the effect of *Platycodon grandiflorum* including platycodin D (PG-Platycodin D) on mast cell degranulation and on the atopic dermatitis-like skin lesion induced by repeated treatment of 2,4-dinitrochlorobenzene (DNCB) on the dorsal skin of ICR mice.
Materials & Methods: mRNA expression was examined by RT-PCR analysis. The efficacy of PG-Platycodin D was tested by scratching behavior, skin severity score, blood IgE level, and histopathological examination.
Results: PG-Platycodin D suppressed the release of β-hexosaminidase, a kind of marker associated with degranulation. PG-Platycodin D inhibited the passive cutaneous anaphylaxis (PCA) reaction in ICR mice efficiently. In addition, molecular analysis demonstrated that PG-Platycodin D inhibited mRNA expression of both IL-3. 10 mg/ml of 5% PG-platycodin D was spread to dorsal directly and sensitized with DNCB for 2 weeks. Histopathologic analysis revealed that thickening of the epidermis and significantly reduced in PG-Platycodin D group. PG-Platycodin D resulted in the suppression of scratching behavior, skin severity score, and blood IgE level.
Conclusion: These results suggest that PG-Platycodin D may be a useful natural resource for the inhibition of atopic dermatitis.

Keywords: PG-platycodin D, atopic dermatitis, DNCB, β-hexosaminidase, passive cutaneous anaphylaxis

Quercetin Induces Apoptosis, Suppresses Migration and Invasion in Human Melanoma Cells through Inhibiting the STAT3 Pathway

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Introduction: Melanoma is highly resistant to chemotherapy and the mortality rate is increasing rapidly worldwide. The STAT3 signaling has been implicated in the pathogenesis of melanoma. Quercetin, a flavonoid molecule ubiquitous in nature, has been shown to have anticancer activities in various types of cancer including melanoma. However, the anti-melanoma mechanisms of quercetin are not fully understood.
Objective: To study the anti-melanoma activity of quercetin *in vitro* and *in vivo* and the involvement of STAT3 in the anti-melanoma effects of quercetin in melanoma A375 and A2058 cells.
Materials & Methods: Antiproliferative and apoptotic effects were detected by MTT assay and PI/Annexin double staining assay, respectively. Protein expression levels were determined by Western blot. Cell scratch assay and Transwell chamber assay were used to determine changes in cell migration and invasion, respectively. Gelatin zymography was used to determine activities of MMP-2 and 9. Nude mice A375 cell xenograft model was used to evaluate the *in vivo* anti-melanoma activity.
Results: Quercetin inhibited melanoma A375 and A2058 cells proliferation; induced apoptosis, which was associated with down-regulation of Mcl-1 expression and up-regulation of cleaved PARP expression; suppressed the migrative and invasive ability through decreasing MMP-2 and 9 activities; reduced constitutive STAT3 phosphorylation and triggered nuclear translocation of STAT3; overexpression of STAT3 rescued quercetin-induced cell death; *in vivo* study showed that quercetin significantly inhibited the growth of xenografted A375 cells.
Conclusion: Quercetin has significant anti-melanoma effect *in vivo* and *in vitro*, the mechanisms may be associated with suppression of STAT3 signaling.

Keywords: quercetin, melanoma, STAT3, migration, invasion

The Anti-inflammatory Potential of *Sutherlandia frutescens*

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Introduction: *Sutherlandia frutescens* is a southern African shrub traditionally used to treat inflammation. Extracts have been shown to decrease glucocorticoid levels in rats subjected to chronic immobilization stress and increase basal levels in non-stressed rats.
Objective: The influence of *S. frutescens* extracts on glucocorticoid biosynthesis and inflammatory gene transcription was investigated.
Materials & Methods: The effects of *S. frutescens* extracts and sutherlandioside B (SUB), a triterpene, on glucocorticoid biosynthesis by cytochrome P450 11β-hydroxylase (CYP11B1) were investigated in COS1 cells. Also, the effects of *S. frutescens* extracts and SUB on steroid production by adrenocortical cells (H295R) were assayed and analyzed by LCMS. Transactivation and transrepression studies were conducted in COS1 cells to determine the effect of the extracts on the glucocorticoid receptor (GR) and subsequent gene activation. Results were visualized using luciferase assays and normalized by protein concentration. MTT assays confirmed cell viability.
Results: *S. frutescens* extracts decreased corticosterone (53%) and cortisol (71%) production by CYP11B1 significantly. The extracts and SUB decreased the steroid metabolites produced by H295R cells. *S. frutescens* extracts did not transactivate gene transcription via the GR in agonist mode. In antagonist mode, the extract increased gene transactivation via the GR. After induction by PMA, *S. frutescens* extracts repressed (40%) the activation of gene transcription via the GR. SUB had no significant effect on inflammatory gene transcription.
Conclusion: *S. frutescens* has potential applications in anti-inflammatory treatment through the ability to reduce glucocorticoid production and repress the activation of inflammatory gene transcription mediated via the GR.

Keywords: *Sutherlandia frutescens*, inflammation, glucocorticoid receptor

Bioaccessible Fraction from *Coccinia grandis* and *Sesbania glandiflora* Inhibits Inflammatory Mediators Producing by Inflamed Caco-2 Cells

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Introduction: *Coccinia grandis* and *Sesbania glandiflora* leaves are common vegetables in Asian diets. Both vegetables were also used as traditional remedy. They contain various phytochemicals and their crude extracts have been previously shown anti-inflammatory and antioxidant activities but their anti-inflammatory activities have never been reported in the enzymatic digested fraction.
Objective: To evaluate anti-inflammatory activity of simulated gastrointestinal digestion (bioaccessible fraction) from *C. grandis* and *S. glandiflora*.
Materials & Methods: Freeze dry samples were simulated gastrointestinal digested and their non-toxic bioaccessible fractions were incubated with human intestinal like Caco-2 cells for 4 h prior to stimulation with IL-1β for 24 h. Culture medium from treated cells were collected to measure TNF-α, IL-6, IL-8 by ELISA. Cell lysates were collected to measure reactive oxygen species (ROS) by spectrofluorometer.
Results: Pre-treated Caco-2 cells with bioaccessible fraction from *C. grandis* and *S. glandiflora* significantly decreased TNF-α, IL-6, IL-8 and ROS producing by inflamed Caco-2 cells.
Conclusion: These data indicated that *C. grandis* and *S. gradiflora* after simulated gastrointestinal digestion effectively attenuated IL-1β induced Caco-2 cells. Nevertheless, these biological functions should be further investigated in inflamed animals and humans.

Keywords: *Coccinia grandi*, *Sesbania glandiflora*, simulated digestion, anti-inflammatory activity

Anti-inflammatory Effect of *Clerodendrum inerme* (L.) Gaertner Leaves in Macrophages Involving the Suppression of NF-κB Activation

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Introduction: *Clerodendrum inerme* (L.) Gaertner is medicinal plants which commonly used in Thai traditional medicine to treat various diseases including inflammation. However, the anti-inflammatory effect of *C. inerme* leaves on the production of inflammatory mediators, nitric oxide (NO) and prostaglandin E_2 (PGE_2) have not been yet determined.
Objective: To examine the inhibitory effects of the extracts of *C. inerme* on NO and PGE_2 production and mechanism underlying its activity on LPS-induced RAW 264.7 macrophages.
Materials & Methods: Anti-inflammatory effect of various fractions (hexane, ethyl acetate and water) of ethanol extract of *C. inerme* leaves was determined from nitrite and PGE_2 levels in macrophage cultured media. The mRNA and protein levels were also determined by real-time reverse transcription-polymerase chain reaction and Western blot analysis, respectively. Bioactive compound from *C. inerme* were isolated by bioassay-guided fractionation technique.
Results: Among solvent extracts, ethyl acetate fraction of *C. inerme* (CIEA) was the most potent inhibitory activity on the production of NO and PGE_2 (IC_{50} value of 32.93 ± 3.95 μg/mL and 25.50 ± 2.13 μg/mL, respectively). CIEA also decreased the mRNA and protein expressions of inducible nitric oxide synthase (iNOS) in LPS-stimulated macrophages. In addition, CIEA suppressed nuclear translocation of NF-κB. From bioassay-guided fractionation of CIEA, *p*-anisic acid was isolated.
Conclusion: The obtained results reveal that leaf extracts of *C. inerme* possess anti-inflammatory properties probably due to the suppression of NO and PGE_2 production. Thus, our data provide scientific evidence that *C. inerme* leaves contain potentially useful agents for treating various inflammatory diseases.

Keywords: *Clerodendrum inerme*, nitric oxide, prostaglandin, anti-inflammatory activity, macrophage

Effect of Cranberry Dietary Supplements with Different Brands on Human CYP3A4 Enzyme

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Introduction: The use of dietary supplements has increased dramatically; making drug interactions with those supplements a major concern. It is remarkable that the rate of herbal usage is much higher in cancer patients. Because dietary supplements are not subject to the same regulations as prescription drugs, we hypothesize that the content of their active ingredients may vary among manufacturers, potentially causing a large variation in therapeutic outcome.
Objective: The present study aimed to test this hypothesis on commonly used cranberry dietary supplements. Activity of human CYP3A4 enzyme was employed as a parameter to determine the effect of cranberry supplement from nine manufacturers.
Materials & Methods: The content of a cranberry product, equal to one capsule, was extracted with methanol. Aliquots of the extract were tested for their ability to inhibit the metabolism of the human CYP3A4 substrate quinine, using an *in vitro* liver microsomal technique. Human liver microsomes and quinine were incubated with or without (i.e. as control) cranberry extract. Formation of quinine's metabolite 3-hydroxyquinine, produced by the CYP3A4-mediated reaction was assayed by a HPLC method.
Results: Of nine cranberry products tested, eight products had little or no effect but only one brand (Nature's Herbs 600 mg) caused very strong inhibition (67.2%) of CYP3A4. The effect of cranberry was varied and ranged from 4.4% activation by Ride Aid 800 mg to 67.2% inhibition by Nature's Herbs 600 mg.
Conclusion: Lack of effect on human CYP3A4 activity suggests that use of cranberry dietary supplement is unlikely to cause significant interactions with drugs metabolized by CYP3A4.

Keywords: dietary supplements, cranberry, CYP3A4, drug interactions

Oyaksungisan, a Traditional Herbal Formula, Suppresses Cell Proliferation by Induction of Autophagy *via* JNK Activation in Human Colon Cancer Cells

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Introduction: Oyaksungisan (OY) is a traditional herbal formula broadly used to treat beriberi, vomiting diarrhea and circulatory disturbance in Asian countries from ancient times. The effect of OY on cancer, however, was not reported until now.
Objective: In this study, we have demonstrated OY inhibits cell proliferation and induces cell death via modulating the autophagy on human colon cancer cells and presented the anti-cancer potential of OY in the treatment of cancer.
Materials & Methods: For preparing OY, twelve medicinal herbs were extracted and lyophilized. The several components of OY were analyzed using HPLC. Cell viability and cytotoxicity were evaluated by MTT assay. The expression of proteins associated with autophagy or apoptosis was confirmed by Western blot analysis. Regulation of MAPK cascades by OY was confirmed using MAPK inhibitors.
Results: In HCT116 cells, OY increased the ratio of LC3–II/LC3–I, a marker of autophagy and its effect was blocked by 3–methyladenine, an inhibitor of autophagy. OY also activated MAPK cascades, especially, JNK activation by OY was significantly related with autophagy effect in HCT116 cells. In contrast, increased cytochrome C release and decreased Bcl–2 level by OY weakly affected activation of caspases and cleavage of PARP in HCT116 cells.
Conclusion: Our results indicate that autophagy induction is responsible for the anti–proliferative effect of OY, despite of weak apoptosis induction in HCT116 cells. Thus, we suggest OY might have a potential to be developed as an herbal anti–cancer remedy.

Keywords: oyaksungisan (OY), autophagy, MAP kinases

Inhibitory Effects of Neem Flowers Extract on Phorbal Ester-induced Expression of Cyclooxygenase-2 and Inducible Nitric Oxide Synthase in Mouse Skin

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Introduction: Neem flowers (*Azadirachta indica* A. Juss) possess a strong chemopreventive potential as well as anticlastogenic activities in laboratory animals.
Objective: The present study was aimed to determine the effect of methanol extract of neem flowers on 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) expression in mouse skin.
Materials & Methods: Female ICR mice were given methanol extract of neem flowers at the doses of 50, 100 and 250 mg/kg body weight by gavage 1 h prior to topical application of TPA (10 nmol) on shaven back skin. Animals were sacrificed after 4 h of TPA application. Treated skin was excised and epidermis was collected by removal of dermis and fat by scrapping. Epidermis was submerged in liquid nitrogen and pulverized using mortar and pestle. Pulverized epidermal tissue was homogenized in cell lysis buffer and protein samples were collected by centrifugation. Collected protein samples were subjected to Western blot analysis.
Results: Methanol extract of neem flowers at the doses of 50, 100 and 250 mg/kg body weight decreased TPA-induced expression of COX-2 and iNOS proteins in mouse skin, but significant reduction was found only in COX-2.
Conclusion: The data indicated that methanol extract of neem flowers at designed doses have inhibitory effect on TPA-induced COX-2 and iNOS protein expression in mouse skin *in vivo*, particularly COX-2.

Keywords: *Azadirachta indica*, TPA, COX-2, iNOS, mouse skin

Phytomedicinal Withanolides Derived from *Withania somnifera* Effectively Block Progression and Metastasis of Breast Cancer by Targeting the Epigenome

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Introduction: Today, the therapeutic use of plant-derived phytochemical compounds in prevention, chemosensitisation or treatment of various chronic inflammatory diseases, including cancer is receiving increased attention in various drug discovery programs worldwide. Increasing evidence suggests that phytochemicals could be used in safe combinations to prevent, reverse and/or block the progression of cancer by targeting multiple pathways in deregulated cancerous cells. The protective activities of natural compounds lie in their ability to modulate the cellular defense mechanisms, including detoxifying and antioxidant enzyme systems, as well as induction of anti-inflammatory, anti-tumor or anti-metastatic responses by targeting specific key transcription factors, cell cycle-related genes, apoptotic genes and/or tumor suppressor or oncogenic genes, which are related to tumor progression. These genes are also known targets of aberrant epigenetic changes, both at the level of DNA methylation and chromatin remodeling.
Objective: We want to map DNA methylation changes upon longterm exposure to Withaferin A *Withania somnifera* which contribute to its anti-cancer activities.
Materials & Methods: We further characterized the anti-cancer activities of Withaferin A by xCelligence assays, invasive assays, FACS analysis, epigenetic cofactor assays, Illumina gene expression array and MBD2-capture sequencing of the DNA methylome.
Results: We show for the first time that the chronic exposure of breast cancer cell lines to low, concentrations (as detected in *in vivo* experiments) of Withaferin A blocks metastatic cancer cell growth and induces epigenetic reprogramming, both at the level of DNA methylation and histone variant gene expression.
Conclusion: Withanolides represent a novel class of anti-cancer compounds with epigenetic modulating properties.

Keywords: withaferin A, cancer-metastasis, DNA-methylome

Effects of *Saussurea involucrata* Extract Flavonoid Molecules Rutin and Its Metabolites on Anti-inflammatory

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Introduction: *Saussurea involucrata* Kar.et Kir., which is a rare traditional Chinese medicinal herb. It has been demonstrated that the *S. involucrata* has the anti-oxidative, antifungal, anti-inflammatory, anti-mutagenic, and anti-neoplastic effects. It has recently been reported that the ethyl acetate fractions of *S. involucrate* (SI-2) could inhibit the proliferation and induced apoptosis in many kinds of solid tumor cells. Rutin (3,3_,4_,5,7-pentahydroxyflavone-3-rhamnoglucoside) and hispidulin are flavonoids of the flavonol type that are found plenty in *S. involucrate*, which had been reported to have anti-inflammation, anti-oxidation and anticancer activity. In previous studies, the gut microflora in the large intestine metabolize rutin to a variety of compounds that include quercetin and phenol derivatives such as 3,4-dihydroxyphenylacetic acid (DHPAA), 3,4-dihydroxytoluene (DHT), 3-hydroxyphenylacetic acid (HPAA), and 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid, HVA).
Objective: This study aimed to evaluate the anti-inflammatory effect of the four metabolites of rutin on lipopolysaccharide (LPS)-stimulated murine macrophages cell lines RAW 264.7 and explore the mechanism of its action.
Materials & Methods: RAW264.7 cells were pretreated with drugs for 30 min prior to incubation with LPS for 24 h. Anti-inflammatory activity was evaluated with reference to iNOS, COX-2, TNF-α and IL-6 gene expression. NO generation were determined by Griess method. The levels of cytokines were determined by enzyme-linked immunosorbent assay (ELISA). In addition, activation of MAPKs and IκB by Western blotting.
Results: Our result showed that 3,4-dihydroxytoluene (DHT) significantly suppresses LPS-induced production of nitric oxide (NO) and expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in a dose-dependent manner, without causing cytotoxicity.
Conclusion: Furthermore, we try to evaluate the 3,4-dihydroxytoluene (DHT) whether reduced LPS-induced nuclear factor κB (NF-κB) and p38 mitogen-activated protein kinases (MAPKs) activation in the further study.

Keywords: *Saussurea involucrata*, flavonoid, 3,4-dihydroxytoluene (DHT), RAW264.7 cell, inducible nitric oxide synthase, cyclooxygenase-2

Compound A Inhibits Cytokine and Chemokine Secretion of DENV-infected HepG2 Cells

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Introduction: A remarkably increased production of cytokines and chemokines, the so called 'cytokine storm', is observed in the patients with dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Inhibition of dengue virus (DENV) replication together with reduction of cytokine and chemokine production may decrease the pathogenesis of DENV infection. Compound A (CpdA) is a dissociated glucocorticoid receptor (GR) ligand, which has a strong anti-inflammatory effect, and may inhibit inflammatory cytokine and chemokine production of DENV-infected cells.
Objective: To test the effect of CpdA on inflammatory cytokine and chemokine production of DENV-infected HepG2 cells.
Materials & Methods: Inflammatory cytokine and chemokine mRNA expression of DENV-infected HepG2 cells was screened by real-time PCR array. The most up-regulated genes were further verified by real-time RT-PCR using different sets of primers. Inhibitory activity of CpdA on cytokine and chemokine production of DENV-infected HepG2 cells was measured by real-time RT-PCR and ELISA, respectively. In addition, leukocyte migration was measured by monocyte chemotaxis assay.
Results: IP-10, TNF-α, and MIP-1β were highly up-regulated in DENV-infected HepG2 cells, respectively. CpdA inhibited the mRNA and protein expression of IP-10, TNF-α, and MIP-1β, and reduced leukocyte migration in a dose-dependent manner.
Conclusion: CpdA can inhibit IP-10, TNF-α, and MIP-1β secretion from DENV-infected HepG2 cells and thereby reduce leukocyte migration.

Keywords: dengue virus, cytokine, chemokine, anti-inflammation, compound A

Glucocorticoid Receptor-dependent Regulation of Skeletal Muscle Mass

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Introduction: Physiological fluctuations of circulating glucocorticoids are known to be important in a variety of biological processes. In fasting conditions, increased secretion of glucocorticoids cause enhanced catabolism of skeletal muscle proteins, resulting in decreased muscle mass. The metabolites of proteins are transported to the liver, being substrates for gluconeogenesis. On the other hand, with abundant nutrition, activation of mammalian target of rapamycin (mTOR) promotes protein anabolism by increasing protein translation rate.
Objective: To elucidate the precise molecular mechanisms for coordinating glucocorticoid actions and mTOR activity in skeletal muscle.
Materials & Methods: We employed the gastrocnemius, soleus, and tibialis anterior muscle from mice as objects of morphological, biochemical, and molecular biological analyses.
Results: We showed that skeletal muscle-specific knockout of glucocorticoid receptor (GR) resulted in significant increase of muscle mass. We identified direct target genes of GR in skeletal muscle, i.e., REDD1 and KLF15. They decreased mTOR activity via a distinct mechanism involving catabolism of branched-chain amino acids, intracellular nutritional activator molecules for mTOR. KLF15 up-regulated the expression of ubiquitin ligases atrogin-1 and MuRF1, and negatively modulated myofiber size. Thus, GR is shown to be a liaison involving a variety of downstream molecular cascades towards muscle atrophy. Notably, mTOR activation inhibited GR transcriptional function and efficiently counteracted the catabolic processes provoked by glucocorticoids.
Conclusion: This mutually exclusive crosstalk between GR and mTOR may be a rational mechanism by which the dynamic balance between catabolic hormone signal and anabolic nutritional signal play a pivotal role in fine-tuning of skeletal muscle mass.

Keywords: glucocorticoid receptor, skeletal muscle, atrophy, atrogenes

Glucocorticoid Receptor (GR)-dependent Gene Regulation in Skeletal Muscle

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Introduction: Skeletal muscle can be mobilized in a number of muscle wasting conditions. Recently, the glucocorticoid receptor (GR) is known to play a role in skeletal muscle metabolism and atrophy, and we identified novel direct target genes of the GR in skeletal muscle, i.e., REDD1 and KLF15. Moreover, we revealed that those GR targets upregulate expression of the atrophy-related genes (atrogenes), which induce to break down skeletal muscle protein to produce amino acids for maintaining energy supply (Cell Metab, 2011).
Objective: To further elucidate the role of GR in skeletal muscle by using skeletal muscle-specific GR knockout mice (GRmKO).
Materials & Methods: GRmKO were generated by breeding GRflox/flox mice with skeletal muscle-specific Cre transgenic mice. We analyzed target gene expressions of the GR in skeletal muscle and plasma corticosterone levels in response to peritoneal injection of dexamethasone and fasting by quantitative RT-PCR and ELISA, respectively.
Results: Western blot analysis revealed that skeletal muscle-specific decrease of GR expression. mRNA expression levels of atrogenes, KLF15, and REDD1 were decreased in skeletal muscle of GRmKO compared with that of control mice (Cont) in response to dexamethasone. After 24 h of fasting, plasma corticosterone levels were increased in both mice and expression levels of atrogenes in skeletal muscle were upregulated in Cont but not in GRmKO.
Conclusion: Not only pharmacological but also physiological concentrations of glucocorticoids are sufficient to elicit significant changes in atrogenes expression in skeletal muscle and the GR in skeletal muscle could play a role in energy homeostasis.

Keywords: glucocorticoid receptor, skeletal muscle, atrophy, atrogenes

Neutralizing TNF Restores GC Sensitivity in a Mouse Model of Neutrophilic Airway Hyperinflammation

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Introduction: Asthma is a heterogeneous disorder characterized by airway hyperresponsiveness and inflammation. Most asthma patients are effectively treated with glucocorticoids (GCs), but some are refractory to the beneficial effects of GCs. Tumor necrosis factor (TNF) is involved in asthma pathology. Therefore, anti-TNF based therapies have been designed, although with conflicting results. However, the effects on steroid resistance were never assessed, as these studies defined their study population by the severity of disease and not by the degree of reversibility after steroid administration.
Objective: We aimed to investigate the role of TNF on the responsiveness to GCs, by using soluble fusion proteins which neutralize TNF effects.
Materials & Methods: We used two different OVA-based mouse models of airway hyperinflammation. The first is GC sensitive and eosinophil-driven, whereas the second represents GC-insensitive, neutrophil-predominant asthma subphenotypes. Different parameters were tested, such as bronchial hyperreactivity, immune cell influx, cytokine synthesis, antibody production and mucus secretion.
Results: TNF blockade restored the beneficial effects of GCs in the GC-insensitive model. Additionally, the impact of TNF on GR actions was studied in bronchial pulmonary A549 cells. We found that TNF attenuates transcriptional activity of GR. We hypothesize that repression of GR transactivation by TNF represents a mechanism for GC insensitivity. We are currently studying the role of several GC-inducible genes in these asthma models.
Conclusion: We demonstrate that TNF reduces the responsiveness to GCs in a mouse model of neutrophilic airway hyperinflammation. Thus, blockade of TNF or a downstream signaling molecule may offer new strategies for therapeutic intervention in GC-insensitive asthma.

Keywords: asthma, glucocorticoid insensitivity, TNF

Role of the Alarmin Interleukin-33 Receptor, ST2, in Inflammatory Responses

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Introduction: Th2-associated ST2 receptor of alarmin interleukin-33 has attracted the attention for its pathogenic role in inflammatory bowel diseases (IBD). *st2* gene is expressed either as a full-length signaling receptor (ST2L) or a soluble (sST2) variant commanded by splicing and alternative distal and proximal promoter activity.
Objective: Due to the relevant implications in inflammatory responses, we studied molecular mechanisms involved in ST2 expression regulation by glucocorticoids (GC), and the association of GC treatment with *st2* gene genetic variants, as predictive clinical evolution factors.
Materials & Methods: By chromatin-immunoprecipitation (ChIP) assays, we studied GR-mediated *st2* gene regulation in mast cells treated with Dexamethasone (DEX). To analyze associating of GC with ST2 expression, IBD patients (163) receiving or not GC treatment were recruited and ST2 levels were determined by ELISA. Clinical and endoscopic characteristics of patients and genetic variants of *st2* gene were analyzed. Un-varied analysis of risk alleles to surgery requirement, extension and disease severity was determined through 2 and contingency analysis.
Results: sST2 expression is induced by GC and GR binding to *st2* promoter increases with DEX and mostly in one GRE, confirming its functionality. In addition, sST2 levels were higher in patients receiving GC compared to other treatment ($p<0.001$). Polymorphic *st2* variant's frequencies were similar between IBD and controls. However, higher ST2 levels and surgery requirement were seen in IBD patients with polymorphic variants (OR = 12.07 -IC = [3.76 - 38.72] - $P<0.0001$).
Conclusion: GR mediates GC-dependent transcriptional *st2* gene activation, through one GRE in distal promoter. A novel GR-dependent mechanism on sST2 induction may contribute to GC anti-inflammatory effects.

Keywords: ST2, glucocorticoids, inflammatory bowel diseases

Selective Modulation of the Glucocorticoid Receptor Can Distinguish between Transrepression of NF-κB and AP-1

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Introduction: Glucocorticoids (GCs) block inflammation via interference of the liganded Glucocorticoid Receptor (GR) with the activity of pro-inflammatory transcription factors NF-κB and AP-1, a mechanism known as transrepression. This mechanism is believed to involve the activity of GR monomers.
Objective: Here, we explored how the GR monomer- favouring Compound A (CpdA) affects the activation and activity of AP-1.
Results: Our results demonstrate that non-steroidal CpdA, unlike classic steroidal GCs, blocks NF-κB but not AP-1-driven gene expression. CpdA rather sustains AP-1-driven gene expression, a result which could mechanistically be explained by the failure of CpdA to block upstream JNK kinase activation and concomitantly also phosphorylation of c-Jun. In concordance and in contrast to DEX, CpdA maintained the expression of the activated AP-1 target gene c-jun, as well as the production of the c-Jun protein. As for the underlying mechanism, ChIP analysis demonstrates that DEX-activated GR, but not CpdA-activated GR, is recruited to AP-1-driven promoters. Furthermore, in mice we observed that CpdA instigates a strong enhancement of TNF-induced AP-1-driven gene expression. Finally, we demonstrate that this phenomenon coincides with an increased sensitivity towards TNF lethality, and implicate again a role for JNK2.
Conclusion: Our data support the hypothesis that a ligand-induced differential conformation of GR may expose different interaction surfaces to yield a different transcription factor cross-talk profile.

Keywords: glucocorticoids, inflammation, dissociated, MAPK, selective GR modulator, c-Jun

Epigenetic Modulation of Oncogenes by Cucurbitacin B in Breast Cancer Cells

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Introduction: Breast cancer is a complex disease driven by multiple factors including both genetic and epigenetic alterations. Recent studies revealed that abnormal gene expression induced by epigenetic changes including aberrant promoter methylation, plays critical role in human breast carcinogenesis. Cucurbitacin B (CuB), an oxygenated tetracyclic triterpenoid compound from Thai medicinal plant *Trichosanthes cucumerina* L., has antiproliferative activity against various human breast cancer cells. The anticancer molecular mechanism of this agent is not completely unveiled.
Objective: In this work, we explore the influence of CuB on the methylation status at promoters of oncogenes *c-myc*, *cyclin-D1* and *survivin* in breast cancer cell lines.
Materials & Methods: The growth inhibitory effect of CuB on breast cancer cells was assessed by MTT assay. Methylation-specific PCR was used to study the methylation pattern of genomic DNA. Real-time RT-PCR and Western blot analysis were performed to determine the mRNA and protein expression levels of all genes studied.
Results: Results indicate that CuB could obviously inhibit cell growth in breast cancer cells. The promoters of oncogenes are usually hypomethylated in cancer cells. Upon CuB treatment, the heavy methylation of these promoters was clearly demonstrated, which consequently downregulated the oncogenic expression of both mRNA and protein levels of all oncogenes studied.
Conclusion: Our results suggest that CuB can effectively inhibit the tumor growth by reversing from DNA hypomethylation to hypermethylation of oncogenes. Hence, cucurbitacin B proves itself as a potential therapeutic agent for cancer, by reversing methylation status of oncogenes, and hence silences the oncogenicity of these genes.

Keywords: cucurbitacin B, hypermethylation, c-Myc, cyclin-D1, survivin

Low 5-methylcytosine Expression as an Aggressive Biomarker for the Urothelial Carcinoma: An Immunohistochemical Study

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Introduction: Previous studies suggested that global DNA methylation is involved in breast, lung, and colon carcinogenesis. However, only a few studies showed the association between global DNA methylation and urothelial carcinoma (UC).
Objective: We constructed a tissue array to elucidate the role of global DNA methylation in UC carcinogenesis.
Materials & Methods: Two tissue microarrays were purchased from US Biomax, Inc. (MD, USA), including 155 tissue cores with 22 normal urothelium samples and 133 urothelium samples with UC. Global DNA methylation (5-methylcytosine; 5-MeC) was measured using the immunohistochemistry (IHC) method (H score) and image analysis (total intensity). Nonparametric analysis with Wilcoxon rank-sum test or the Kruskal–Wallis test was applied to compare the differences in 5-MeC levels and the clinical variables between the two groups.
Results: The results indicated that 5-MeC staining expression (either H score or total intensity) was significantly lower in the urothelium samples with UC than that in normal urothelium. Urothelium samples with advanced stage showed significantly lower 5-MeC staining expression than those with early stage. The 5-MeC staining expression did not show any difference in terms of gender or tumor grade.
Conclusion: The 5-MeC levels measured by IHC might be a good method for clinicians to evaluate global DNA methylation in UC progression.

Keywords: global DNA methylation, immunohistochemistry, urothelial carcinoma, 5-methylcytosine

Phytomedicinal Withanolides Derived from *Withania somnifera* Effectively Block Progression and Metastasis of Breast Cancer by Targeting the Epigenome

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Introduction: Today, the therapeutic use of plant-derived phytochemical compounds in prevention, chemosensitisation or treatment of various chronic inflammatory diseases, including cancer is receiving increased attention in various drug discovery programs worldwide. Increasing evidence suggests that phytochemicals could be used in safe combinations to prevent, reverse and/or block the progression of cancer by targeting multiple pathways in deregulated cancerous cells. The protective activities of natural compounds lie in their ability to modulate the cellular defense mechanisms, including detoxifying and antioxidant enzyme systems, as well as induction of anti-inflammatory, anti-tumor or anti-metastatic responses by targeting specific key transcription factors, cell cycle-related genes, apoptotic genes and/or tumor suppressor or oncogenic genes, which are related to tumor progression. These genes are also known targets of aberrant epigenetic changes, both at the level of DNA methylation and chromatin remodeling.

Objective: We want to map DNA methylation changes upon longterm exposure to Withaferin A *Withania somnifera* which contribute to its anti-cancer activities.

Materials & Methods: We further characterized the anti-cancer activities of Withaferin A by xCelligence assays, invasive assays, FACS analysis, epigenetic cofactor assays, Illuminagene expression array and MBD2-capture sequencing of the DNA methylome.

Results: We show for the first time that the chronic exposure of breast cancer cell lines to low, concentrations (as detected in *in vivo* experiments) of Withaferin A blocks metastatic cancer cell growth and induces epigenetic reprogramming, both at the level of DNA methylation and histone variant gene expression.

Conclusion: Withanolides represent a novel class of anti-cancer compounds with epigenetic modulating properties

Keywords: withaferin A, cancer-metastasis, DNA-methylome

Parallel Conference

Immunotherapy of Cancer, Hepatitis C and Allergy

December 21st, 2012
Srisavarindira Building
Faculty of Medicine Siriraj Hospital,
Mahidol University, Bangkok, Thailand

MMC2012 Parallel Conference

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Scientific Program

Friday

December 21st, 2012

13:00-13:45	**"Immunotherapy of Cancers"** Professor Lung-Ji Chang, Department of Molecular Genetics and Microbiology, University of Florida, Florida, USA
13:45-14:15	**"Therapeutic Nanobody for HCV Infection"** Dr. Jeeraphong Thanongsaksrikul, Faculty of Allied Health Sciences, Thammasat University, Thailand
14:15-14:45	**"Cell Penetrable Humanized-VH/V_HH that Inhibit RNA Dependent RNA Polymerase (NS5B) of HCV"** Dr. Kanyarat Thueng-in, Faculty of Veterinary Medicine, Kasetsart University, Thailand
14:45-15:00	Coffee Break
15:00-15:45	**"Allergen Immunotherapy for Allergic Disease"** Assoc. Prof. Pongsakorn Tantilipikorn, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand
15:45-16:15	**"Allergenome of *Vespa affinis*"** Asst. Prof. Nitat Sookrung, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand
16:15-17:00	**"Cockroach Allergy and Therapeutic Vaccines"** Professor Wanpen Chaicumpa, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand

Invited Lectures

Immunotherapy of Cancers

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Introduction: Hematopoietic stem cells (HSCs) have been used to reconstitute the immune system in cancer patients who receive chemotherapy or radiation therapy. Complementary to surgery, chemotherapy and radiation therapy, immunotherapy has proven to be effective against both solid tumors and cancers of the blood. Bone marrow transplant is an extreme form of immunotherapy that is based on stem cells and immune cells of a donor (allograft). Autotransplant, on the other hand, is based on patient's own bone marrow stem cells and immune cells (autograft), which is routinely applied in the clinics.
Objective: To discuss the current state of T cell-based therapies involving innovative immune modulation and genetic engineering of the immune effector cells.
Materials & Methods: Cancer cells, including surgical specimens and commercial cell lines, were obtained under institutional review board (IRB) approval or material transfer agreement. Immune cells including dendritic cells as well as T cells were obtained from patients in the clinics or blood donation center with IRB approvals. A highly efficient gene delivery system based on lentivirus has been developed in my laboratory. The lentivectors have been used to modify cancer cells, to enhance tumor immunogenicity, to modify dendritic cells with increased antigen presentation and T cell stimulatory functions, and to modify T cells to generate major histocompatibility complex (MHC) unrestricted target-specific killer cells.
Results: Lentiviral vector-modified cancer cells or dendritic cells displayed increased antigen stimulation functions that effectively activated T cells *in vitro* to generate antigen-specific effector cells. Immune modulation through lentiviral modification is key to the increased targeting and killing functions of the T cells. Importantly, cancer patients often develop a strong tolerance toward cancer antigens, which can be readily demonstrated *in vitro* using immune cells derived from cancer patients. Therefore, *in vitro* isolation and expansion of cancer-specific T cells do not always guarantee the success of cancer immunotherapy. Alternatively, engineering of T cell receptor (TCR) with antigen-specific single chain antibody gene can direct the functional T cells to target cancer cells expressing the specific surface antigens. This latter approach allows for MHC-independent T cell therapy to be applied in a short period of time without lengthy *in vitro* handling. Further safety improvement in the engineering of the chimeric TCR will be key to rapid translation of this powerful technology to the clinics in the near future.
Discussion: Improved lentiviral vector system, dendritic cell and T cell culture systems, and advanced chimeric TCR engineering strategies will be presented and discussed.
Conclusion: The current lentiviral vector and immune cell based technologies provide a highly innovative anti-viral and anti-cancer therapy that may revolutionize modern medicine.

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Therapeutic Nanobody for HCV Infection

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Introduction: Antibody library containing phages that display humanized-camel nanobodies (VH/V_HH) was established. Several nanobodies that interfered with biological activities of enzymes, toxins, and other functionally different proteins were produced from the VH/V_HH library. The antibodies preferentially recognized conformational structure of the targets. They inhibited the toxins/enzymes by inserting CDR loops into the active grooves and directly blocked the activities. Currently, p7 viroporin, one of hepatitis C virus (HCV) proteins, has become an attractive target of anti-HCV agents. The p7 ion channel activity is essential for assembly and release of new virus particles. Therefore, humanized-nanobodies that can traverse across cell membrane into HCV infected cells and react specifically to- and inhibit the function of- the p7 should have high potential as a novel anti-HCV agent.
Objective: To produce cell penetrable, p7-specific humanized-nanobodies that interfere with the egress of HCV from infected cells.
Materials & Methods: Synthetic peptide derived from p7 of HCV 3a was used in phage biopanning for selecting phage clones that displayed the nanobodies (VH/V_HH) bound to p7 from a humanized-camel VH/V_HH display library. Antigenic specificity of the nanobodies was confirmed by indirect ELISA and *in situ* immunohistochemistry. The genes coding for the antibodies of interest were molecularly linked with a DNA sequence coding for a cell-penetrating hydrophobic peptide, penetratin, to produce cell penetrable nanobodies or transbodies. HCV RNA released from the virus infected Huh7 cells that had been incubated with the transbodies and grown in the medium replenished with the transbodies were determined by using qRT-PCR.
Results: Humanized, cell penetrable transbodies specific to p7 viroporin derived from some recombinant *vh/v_hh*-vector transformed *E. coli* clones could reduce the amounts of HCV RNA in the spent medium of Huh7 cells infected with the antibody exposed-HCV, implying that the transbodies could inhibit the egress of the newly produced virions.
Discussion & Conclusion: The molecular mechanism of inhibition of the HCV egress mediated by the cell penetrable p7-specific nanobodies needs elucidation.

Acknowledgements: This work was supported by the Thailand Research Fund (DPG5380001) and The National Research University (NRU) Project, Office of Commission on Higher Education, Ministry of Education, Thailand.

Keywords: P7 viroporin, hepatitis C infection, therapeutic antibody, single domain antibody, nanobody, cell penetrable antibody

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Cell Penetrable Humanized-VH/V_HH that Inhibit RNA Dependent RNA Polymerase (NS5B) of HCV

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Introduction: The NS5B protein has RNA-dependent RNA polymerase (RdRp) activity which is pivotal for *de novo* RNA synthesis of hepatitis C virus (HCV). The protein is an attractive target of developing anti-HCV agents.
Objective: This work aimed to produce cell penetrable humanized single domain antibodies (SdAb; VH/V_HH) that interfere with the RdRp activity.
Materials & Methods: Recombinant NS5BΔ55 of genotype 3a HCV with *de novo* RNA synthetic activity was produced and used in phage biopanning for selecting phage clones that displayed NS5BΔ55 bound VH/V_HH from a humanized-camel VH/V_HH display library. Indirect ELISA and Western blot were used to determine the binding specificity of VH/V_HH against recombinant NS5BΔ55. NS5BΔ55 activity and VH/V_HH inhibit NS5BΔ55 activity were determined by ELISA using 3'di-cytidylate 25 nucleotide directed *in vitro* RNA synthesis. The selected VH/V_HH were linked molecularly to a cell penetrating peptide, penetratin. The amounts of RNA intracellularly and in culture medium of JFH-1HCV RNA transfected Huh7 cell after incubated with VH/V_HH were determined by using real time RT-PCR.
Results: VH/V_HH from *E. coli* transfected with four selected phage clones inhibited RdRp activity. The cell penetrable VH/V_HH added to culture of Huh7 cells transfected with JHF-1 RNA reduced the amounts of RNA intracellularly and in culture medium implying that they inhibited the virus replication.
Discussion & Conclusion: This study is the first report on HCV polymerase neutralization by cell penetrable humanized-VH/V_HH. The transbodies await further studies for *in vivo* role in inhibiting HCV replication.

Acknowledgements: This work was supported by the Thailand Research Fund (DPG5380001) and The National Research University Project, Office of Commission on Higher Education, Ministry of Education, Thailand.

Keywords: RNA dependent RNA polymerase, hepatitis C infection, therapeutic antibody, single domain antibody, phage bio-panning, *de novo* RNA synthesis

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Allergen Immunotherapy for Allergic Disease

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Allergen immunotherapy (AIT) is a method of gradually administration of allergen(s) into the body of allergic patients. The allergen should be the allergen which responsible for causing allergic symptoms in the particular individual. For this reason, AIT can be called as: Specific Immunotherapy (SIT).

AIT is one of the standard modalities for allergic rhinitis, allergic asthma and venom hypersensitivity. Its role in allergic skin disease and food allergy is still uncertain. AIT plays a crucial role in the modification of the T-cell response especially T-regulatory cell (Treg). It can cause the immune deviation for T-helper 2 (Th2) to Th1. These mechanisms lead to the patients' immune tolerance to allergens.

Conventional AIT is the method of subcutaneous immunotherapy (SCIT) into the body. The schedule of SCIT takes approximately 3-6 months to reach the desired dose of maintenance. This phase is called induction phase. After the patients reach the maintenance (effective) dose, the schedule can be made for every 4 to 6 weeks. Some modifications of injection schedule are the RUSH or Cluster schedules which shorten the induction phase into one week or 2 months.

Even though the SCIT has been approved for its efficacy by many meta-analysis, it may cause inconvenient and discomfort to the patients. Another route of administration is the sublingual immunotherapy (SLIT). SLIT causes almost similar immunologic mechanism to immune tolerance as SCIT. It also gains the excellent effectiveness and, moreover, safety. But its drawback is the availability of allergens and the opportunity of mixing allergen in the multi-sensitization patients.

The update mechanism of both SCIT and SLIT will be presented. Also the evidence base of both AITs method for each allergen will be discussed as well.

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Keywords: allergic disease, immunotherapy

Allergenome of *Vespa affinis*

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Introduction: Wasp venom allergy is an IgE-mediated (type-1) hypersensitivity. In Thailand, the predominant wasp species causing the problem is *Vespa affinis* (Tor Hua Suea). The clinical patterns of the wasp sting may be local or severe systemic reaction leading to anaphylactic shock, acute renal failure and eventually death. Information on wasp venom allergy, including the venom biological nature, molecular structure and physiological functions as well as their role as allergens among patients are scarce.
Objective: This study aimed to identify all proteins and allergenome in the holovenom of *Vespa affinis*.
Materials & Methods: The venom sacs were removed from the sting apparatus by pulling them out of the bodies using forceps and small scissors. All proteins in the holovenom were verifiled by two dimensional gel electrophoresis and mass spectrometry. Reactivities of the proteins to IgE in sera of patients allergic to *V. affinis* venom were determined by IgE immunoblotting.
Results: Venomics of adult *Vespa affinis* were revealed as 94 proteins by two-dimensional gel electrophoresis (2DE)-based proteomics. Among them, 66 protein spots could be identified by LC-MS/MS; however, no peptides of the database matched with peptides derived from the other 28 venom proteins. 2DE-IgE immunoblotting for studying wasp venom allergenome (wasp components bound by IgE in allergic patients' sera) showed that four major allergenic components were bound by serum IgE of more than 50% of the wasp allergic Thai patients. These components were phospholipase A1 (100%), GB19860 transcription protein (76.9%), enolase (61.5%), and venom allergen-5 (61.5%). Some patients had specific IgE to wasp minor allergens including hyaluronidase (46.1%) and arginine kinase-like protein (46.1%).
Conclusion: The results obtained from this study gives insight into the venomics and the allergenic, both major and minor, components of *Vespa affinis*. The major allergenic role of the wasp PLA1 is demonstrated for the first time. The allergenic proteins reported in this study have potential application as therapeutic vaccine components for wasp venom specific immunotherapy.

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Keywords: *Vespa affinis*, venomics, allergenomics, two-dimensional gel electrophoresis, phospholipase A1

Cockroach Allergy and Therapeutic Vaccines

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Introduction: Cockroach (CR) allergens frequently cause severe asthma in CR sensitized subjects. The pharmacologic treatment of allergic asthma is purely symptomatic and does not address the underlying immunologic mechanisms. Allergen specific immunotherapy (IT) causes a shift of allergic Th2 responses towards Th1 and Treg responses which reduce airway inflammation and prevent disease progression.
Objective: In this study, the therapeutic efficacy of an intranasal liposome-adjuvant vaccine made of a refined major allergen of *Periplaneta americana*, i.e., arginine kinase (AK) or Per a 9, was compared to the liposome entrapped-*P. americana* crude extract (CRE) vaccine.
Materials & Methods: Adult BALB/c mice were rendered allergic to CRE. They were divided into 3 groups and were immunized intranasally on every alternate day with 8 doses of liposome entrapped-CRE (L-CRE), -AK (L-AK) and placebo (liposome entrapped PBS, L-P), respectively. One week later, all mice received a nebulized CRE provocation. Vaccine efficacy evaluation was performed one day post-provocation.
Results: Liposome entrapped-native AK could attenuate airway inflammation after the CRE provocation and caused a shift of allergic Th2 to Th1 and Treg responses. The L-CRE also induced a shift from the Th2 to the Th1 response but did not induce a Treg response and could not attenuate the airway inflammation upon allergen re-exposure.
Discussion & Conclusion: The findings of this study, not only document a more comprehensive and beneficial immune response induced by L-AK, compared to L-CRE and a placebo, but also raise the point that the shift from the Th2 to the Th1 response alone might not correlate with an improved airway histopathology, clinical outcome and quality of life.

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Keywords: allergen specific immunotherapy, cockroach allergy, intranasal vaccines, liposome, Per a 9 (arginine kinase), *Periplaneta americana*, Th1/Th2/Treg, cytokines

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Poster Presentations

Production of Humanized-camel VH/V_HH that Neutralizes NS3 Helicase Activity of Hepatitis C Virus

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Introduction: Hepatitis C virus (HCV) is a major cause of chronic liver diseases which may progress to end stage liver diseases. Combined pegylated-interferon with nucleoside analog, ribavirin, is used for treatment of HCV infection. There are drawbacks of the treatment protocol including long duration, adverse side effects, drug resistant HCV genotypes and high cost. There is a need of new anti-HCV agents especially those that can cope up with the limitations of the existing therapeutic regimen.

Objective: To produce cell penetrable humanized-camel single domain antibodies (VH/V_HH) that bound specifically to the HCV NS3 protein and can neutralize the protein helicase activity.

Materials & Methods: Recombinant NS3 protein (rNS3) was produced from HCV genotypes 3a (common genotype) and used in phage bio-panning for selecting phages displaying rNS3 bound-VH/V_HH from an established humanized-camel VH/V_HH phage display library. *E. coli* were transfected with the phages and screened for the clones that expressed the single domain antibodies. Soluble VH/V_HH antibodies that bound specifically to the rNS3 helicase were tested for their ability to neutralize the HCV helicase activity *in vitro* by gel-based-assay and FRET. Gene sequences coding for the VH/V_HH of interest were subcloned into plasmid backbone with inserted DNA coding for a cell penetrating peptide, penetratin (PEN). The recombinant plasmids were put in *E. coli* and the cell penetrable VH/V_HH anibodies were produced from the plasmid transformed *E. coli* factories.

Results: Recombinant NS3 helicase of HCV genotype 3a was produced successfully. Four phagemid transformed *E. coli* produced soluble VH/V_HH antibodies that exhibited significant inhibitory activity on rNS3 helicase mediated unwinding of synthetic dsDNA substrate. Gene sequences coding for the VH/V_HH were subcloned readily into *pen*-pETb23+ plasmids. Cell penetrable VH/V_HH could be produced and purified from the respective *E. coli* transfected with the *pen-vh/*v_h*h*-plasmids.

Discussion & Conclusion: The so-produced HCV helicase specific cell penetrable humanized-camel VH/V_HH antibodies will be tested for their ability to inhibit HCV replication in HCV infected hepatic cells. The single domain antibodies that give promising result should be tested further in clinical trials for the ultimate purpose of using them as a novel and broad spectrum anti-HCV.

Acknowledgements: This work was supported by the Thailand Research Fund (DPG5380001) and the National Research University (NRU) Project, Office of the Commission on Higher Education (CHE), Ministry of Education, Thailand. We thank Professor Dr. Yong Poovorawan, Chulalongkorn University, Bangkok, Thailand, for the supply of genotype 3a HCV infected sera. Thanks are due to Dr. Takaji Wakita, Department of Microbiology, Tokyo Metropolitan Institute for Neuroscience, Tokyo, Japan and Professor Dr. Ralf Bartenschlager, Department of Molecular Virology, University of Heidelberg, Germany, for the supply of the genotype 2a HCV JFH-1 plasmid.

Keywords: hepatitis C virus (HCV), helicase, NS3-C, humanized-camel VH/V_HH, penetratin (PEN)

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Humanized Single Domain Antibodies (VH/V_HH) that Bound Specifically to *Naja kaouthia* Phospholipase A_2 and Neutralized the Enzymatic Activity

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Introduction: *Naja kaouthia* (monocled cobra) venom contains many isoforms of secreted phospholipase A_2 ($sPLA_2$). The $sPLA_2$ exerts several pharmacologic and toxic effects in the snake bitten subjects, both dependent and independent on the enzymatic activity.
Objective: To produce prototype humanized-VH/V_HH that can neutralize the *N. kaouthia* $sPLA_2$ enzymatic activities for developing further into an adjunctive and safe therapeutic agent against the snake venoms.
Materials & Methods: Secreted PLA_2 of *N. kaouthia* was purified from the holovenom by ion exchange column chromatography. The enzymatic activity was determined by using $sPLA_2$ assay kit. Phage clones displaying humanized-camel single domain antibodies (VH/V_HH) that bound to the $sPLA_2$ were selected from a VH/V_HH phage display library, introduced into suppressor *E. coli*, and the VH/V_HH antibodies were purified from the lysates of transformed *E. coli* grown under IPTG induction condition. The antibodies were screened for specific binding to the $sPLA_2$ by using indirect ELISA and Western blotting. *N. kaouthia* $sPLA_2$ was mixed with purified VH/V_HH and the PLA_2 inhibitory activities of the antibodies were calculated in comparison to the $sPLA_2$ incubated with the PBS alone.
Results: *N. kaouthia* $sPLA_2$ appeared in two protein profiles, P3 and P5, after fractionation of the holovenom. The P3 and P5 catalytic activities were 0.47 and 0.13 μmol/min/ml, respectively. VH/V_HH from lysates of three *E. coli* clones, V_HH-P3-1, V_HH-P3-3 and V_H-P3-7, bound to the P3 and the P5. The antibodies when mixed with the P3 and P5 at molar ratio 3:1 and 5:1 inhibited the PLA_2 activity by 32, 52 and 16% and 19, 37 and 26%, respectively. The VHH-P3-3 exerted the best neutralizing activities which were similar to the horse antivenin used currently for treatment of the cobra bitten subjects.
Conclusion: Humanized-camel single domain antibodies (VH/V_HH) that neutralized enzymatic activity of different isoforms of *N. kaouthia* secreted PLA_2 were produced successfully. They should be developed further into the real use for safe and effective therapy of cobra bites.

Acknowledgements: The work was supported by the National Research University (NRU) Project of the Office of the Commission on Higher Education (CHE) and the Thailand Research Fund (TRF) through DPG5380001 grant. Charnwit Chavanayarn is the NRU project scholar. Nitat Sookrung is the TRF new scholar.

Keywords: snake venom, *Naja kaouthia*, phospholipase A_2 (PLA_2), single domain antibody (SdAb), VH/V_HH

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Cell Penetrable Human ScFv Specific to Influenza A Virus Matrix Protein, M1, Mitigates Influenza Severity

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Introduction: Matrix protein-1 (M1) is highly conserved across type A influenza viruses. This protein has many important functions in the viral replication cycle; thus it is one of the targets of novel anti-influenza.
Objective: To study therapeutic efficacy of M1 specific, cell penetrable human single chain antibody (HuScFv) in mitigating severity of influenza in infected mice.
Materials & Methods: *E. coli* derived-human single chain antibody fragments (HuScFv) specific to recombinant M1 of influenza A virus H5N1 (clade 1) was produced using a human antibody phage display library. HuScFv from selected *huscfv*-phagemid transformed *E. coli* clones were linked molecularly to a cell penetrating peptide, penetratin (PEN) and PEN-HuScFv were produced and purified. BALB/c mice were infected intranasally with mouse adapted-avian H5N1 virus (clade 2.3). They were then treated with M1 specific-PEN-HuScFv. Control infected mice received PBS treatment. Internal organs (lung, brain, spleen, liver and kidney) were collected. Tissue viral loads were determined by real time RT-PCR. Histopathology of the tissues was also examined.
Results: Infected BALB/c mice that received M1 specific-PEN-HuScFv had reduced viral loads and histopathological features in tissues compared to the controls.
Discussion & Conclusion: M1 specific-PEN-HuScFv could mitigate severity of influenza in mice infected with A H5N1 of the heterologous clade. Cross therapeutic efficacy of the transbodies on influenza caused by different virus subtypes remains to be evaluated.

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Keywords: cell-penetrating antibody, influenza viruses, matrix protein-1 (M1), phage display, qPCR

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Humanized Single Domain Antibodies (VH/V_HH) that Bound Specifically to Non-structural Protein 4B (NS4B) of Hepatitis C Virus

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Introduction: Non-structural protein 4B (NS4B) of hepatitis C virus (HCV) is believed to be involved in multiple steps of the viral RNA replication including membrane web formation, RNA binding and NTP hydrolysis activity. Therefore, the HCV NS4B is a suitable target of anti-HCV remedy.
Objective: This study aimed to produce cell-penetrable humanized-camel single domain antibody fragment (VH/V_HH) that bind specifically to HCV NS4B and interfere with the NS4B activity for the ultimate purpose of developing further for treatment of HCV infection.
Materials & Methods: HCV NS4B coding sequence was amplified by using cDNA of JFH1 HCV as template. Recombinant NS4B was produced and used in phage bio-panning to select phage clones that displayed NS4B-bound VH/V_HH from the humanized-camel antibody phage library. Soluble VH/V_HHs were expressed from the *vh/vhh*-phagemid transformed *E. coli* and determined the binding to the homologous antigen by indirect ELISA and Western blotting. Immunoglobulin domains of the NS4B-bound VH/V_HH were identified by IMGT server. Their complementarity determining regions (CDRs) and immunoglobulin frameworks (FRs) were determined.
Results: Recombinant HCV NS4B protein was successfully produced and used in phage bio-panning. Twenty clones of *vh/vhh*-phagemid transformed *E. coli* could express soluble VH/V_HHs. All of the soluble VH/V_HHs bound specifically to the HCV NS4B.
Discussion & Conclusion: The so produced HCV NS4B-specific humanized-camel VH/V_HHs will be developed into cell penetrable format and evaluated further for their ability to neutralize the NS4B biofunctions as well as ability to inhibit HCV replication.

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Keywords: VH/V_HH, NS4B, phage display, single domain antibody, hepatitis C virus, viral hepatitis

Mouse Models of Allergies to Cat and Dog Allergens

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Introduction: Cat and dog contribute a rich source of airborne allergens. Their major allergens: Fel d 1 and Can f 1, respectively, are from dander and saliva. Allergen specific immunotherapy is the only measure that cures/prevents disease progression by causing a shift of allergic/pathogenic Th2 towards Th0/Th1 and/or regulatory T cell responses.
Objective: To develop mouse models of cat and dog allergies for use in efficacy testing of immunotherapeutic vaccines
Materials & Methods: PBST washed hair/dander samples of healthy cats and dogs were sonicated filtered, centrifuged. The clear supernatants (crude hair/dander extracts, CEs), were dialyzed against distilled water and lyophilized. BALB/c mice were sensitized intraperitoneally with 150 µg CEs mixed with alum on days 0, 7 and 14, intranasally (CEs alone) on days 21, 23 and 25, and nebulized on days 32, 33 and 34 with 10 mg CEs in 10 ml PBS. Sera were collected on day 35 for allergen specific IgE determination by indirect ELISA. Bronchoalveolar lavage fluids (BALF) and lung tissues were used for inflammatory cell enumeration and histopathology study, respectively. Sham mice received PBS at the same timeline as allergenized mice.
Results & Discussion: Inflammatory cells (eosinophils, neutrophils) in BALF of only some allergenized mice were higher than normal and sham mice. Some allergenized mice showed moderate severity of lung histopathology and specific IgE increment.
Conclusion: There was a tendency that cat and dog CEs could induce allergy in the allergenized mice. For stronger allergic features, higher CE dosage and longer sensitization duration are needed.

Acknowledgements: The work was supported by the National Research University (NRU) Project of the Office of Commission on Higher Education (CHE) and Thailand Research Fund (DPG5380001).

Keywords: Fel d 1, Can f 1, pet dander allergy, allergy models

Production of HuScFv and VH/V_HH for Inhibiting HCV Serine Protease Activity

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Introduction: NS3-4A protease is an essential component of the viral replication complex and act as a key player in persistence and pathogenesis of hepatitis C virus (HCV). The HCV NS3-4A is a therapeutic target of anti-HCV agents as the protein is responsible for cleaving the viral polyprotein and the key host-adapter proteins MAVS and TRIF, which modulates growth factor signaling and block the innate immune pathway. Blocking of the HCV NS3-4A protease could limit the processing of the HCV polyprotein resulting in interference with the virus replication.
Objective: The objective of this study was to produce human single chain variable antibody fragments (HuScFv) and humanized-camel single domain antibodies (VH/V_HH) that neutralize HCV NS3 protease (NS3-4A) activity for use in HCV replication inhibition.
Materials & Methods: Recombinant NS3-4A (rNS3-4A) of HCV genotype 3a was generated and affinity-purified. The serine protease activity of rNS3-4A was determined by Fluorescence Resonance Energy Transfer (FRET) assay. The rNS3-4A was used in biopanning for selecting phages that display the antigen bound human single chain variable fragment antibodies (HuScFv) and humanized-camel single domain antibodies (VH/V_HH) from the in-house established libraries. The so produced rNS3-4A-specific HuScFv and VH/V_HH were determined for homologous antigen binding by ELISA and Western blotting. The antibodies were pre-incubated individually with the rNS3-4A, added with FRET reaction mixture and measured the fluorescent signal by spectrofluorometer.
Results: The HuScFv and VH/V_HH antibodies that bound specifically to HCV NS3-4A and reduced the serine protease activity of the HCV protein were successfully produced.
Discussion & Conclusion: HuScFv/VH/V_HH which showed promising results on inhibition of the NS3-4A protease activity will be evaluated further for their ability to inhibit HCV replication by *ex vivo* neutralization assay.

Acknowledgements: This work was supported by the Thailand Research Fund (DPG5380001) and The National Research University Project, Office of the Commission on Higher Education, Ministry of Education,Thailand. Jittavisutthikul S. is a Royal Golden Jubilee (RGJ) Ph.D. scholar of the Thailand Research Fund. We thank Professor Yong Poovorawan, Chulalongkorn University, Bangkok, Thailand, for the supply of genotype 3a HCV infected sera. Thanks are due to Dr. Takaji Wakita, Department of Microbiology, Tokyo Metropolitan Institute for Neuroscience, Tokyo, Japan and Professor Dr. Ralf Bartenschlager, Department of Molecular Virology, University of Heidelberg, Germany, for the supply of the genotype 2a HCV JFH-1 plasmid.

Keywords: NS3-4A protease, hepatitis C virus, human ScFv, humanized-VH/V_HH, phage display

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Human ScFv That Neutralizes Biological Activity of HIV-1 Vpu Protein

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Introduction: Vpu is a multifunctional protein which is pivotal for HIV growth and spread and also the progression of the infection to AIDS. Thus, specific inhibition of the *in vivo* bioactivities of Vpu would ultimately interfere with the HIV infectious cycle.
Objective: This study aimed to produce fully human monoclonal single chain antibody (HuScFv) that bound specifically to Vpu for developing further to anti-HIV-1 agent.
Materials & Methods: Recombinant Vpu protein (rVpu) was produced from HIV-1 (CRF_AE) by molecular cloning techniques. The recombinant protein was used as panning antigen for selection of phage clones that displayed Vpu bound-HuScFv from a HuScFv phage display library constructed previously. Antigenic specificity of HuScFvs against Vpu protein derived from lysates of *E. coli* transfected with the Vpu bound phages were tested by indirect ELISA and Immunoblotting. The HuScFvs were made into cell-penetrable format by molecular linking the respective *huscfv* sequences to DNA sequence coding for a 16 amino acid cell penetrating peptide, penetratin (PEN) in the *pen*-pET23b+ plasmid backbone. The PEN-HuScFv antibodies were prepared from the *E. coli* transfected with the *pen-huscfv*-plasmids. The DNA sequences coding for the PEN-HuScFvs were sequenced and deduced into amino acid sequences by using IMGT database.
Results: The recombinant Vpu protein was successfully produced and purified for use in the phage biopanning process. HuScFvs of 5 clones bound specifically to the Vpu as determined by indirect ELISA and Immunoblotting. All HuScFvs revealed complete VH and VL coding sequences that could be deduced individually into four immunoglobulin frameworks (FRs 1-4) and three complementarity determining regions (CDRs 1-3) of each antibody variable domain (VH/VL). The HuScFv coding sequences (*huscfvs*) were subcloned successfully into the *pen*-pET23b+ plasmids and the cell-penetrable HuScFvs (PEN-HuScFvs) were produced from the *pen-huscfv*-plasmid transformed *E. coli* clones.
Conclusion: To our knowledge, this is the first report on production of cell penetrable human single chain antibody fragments that specifically bound to the Vpu protein of HIV-1. The cell penetrable HuScFvs will be tested further for their ability to interfere with the Vpu biofunctions and the HIV replication inhibition.

Acknowledgements: The work was supported by the National Research University (NRU) project of the Office of Commission on Higher Education (CHE) and the Thailand Research Fund (TRF) through DPG5380001 grant. Nitat Sookrung and Nitaya Indrawattana are TRF new scholars of the mrg2554 and the mrg2555, respectively.

Keywords: AIDS, HIV-1, Vpu, cell-penetrable human ScFv, phage display

Human Monoclonal Antibodies That Bind Specifically to- and Interfere with the Bio-functions of Influenza Virus Surface Exposed Proteins; Hemagglutinin-1 and M2 Proteins of A/H1N1 2009

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Introduction: The hemagglutinin and matrix 2 are proteins which expose on the envelope membrane of influenza virus therefore these proteins are vulnerable target for novel neutralizing antibodies.
Objective: To produce human single chain variable antibody fragments (HuScFv) that bind specifically to- and interfere with the bio-functions of influenza virus surface exposed proteins; hemagglutinin-1 and M2 proteins for use as a sole or adjunct therapeutic agent for human influenza.
Materials & Methods: A/Thailand/CU-H106/2009 (H1N1) was propagated in embryonated eggs and inactivated by 10% formalin. The inactivated virus particles adsorbed onto human red blood cell ghosts were used as antigen in selecting phage clones displaying HuScFv from a human antibody phage display library that had been subtracted with the packed human red blood cell ghosts. The phages bound to the virus adsorbed on the red blood cell ghosts were added with *E. coli* host. After allowing phage transfection into *E. coli*, the bacterial clones carrying *huscfv*-phagemids were screened and the clones that could express soluble HuScFv were selected. Antigenic specificities of the HuScFv purified from several *E. coli* transformants were determined by ELISA and Western blotting using recombinant full length H1, N1, and M2 influenza A proteins as antigens. DNA banding patterns of each *huscfv* was determined by using restriction fragment length polymorphism (RFLP) experiment to reveal the diversity of *huscfv*. The HuScFv bound to H1 and M2 were tested for their ability to inhibit influenza replicative cycle *ex vivo* in influenza infected MDCK cells.
Results: Soluble specific HuScFv to native H1 and M2 was produced and purified from transformed *E. coli* clones. The RFLP experiments also revealed multiple DNA banding patterns which indicated epitope/affinity diversity of the HuScFv. The selected HuScFv showed inhibition in influenza replicative cycle *ex vivo* by using described method.
Conclusion & Recommendation: Soluble HuScFv to H1 and M2 of A/H1N1 2009 influenza virus that inhibit influenza replicative cycle were successfully produced from *E. coli* transformants carrying *huscfv*-phagemids. The HuScFv warrants testing further for their *in vivo* protective efficacy in mammalian models of influenza such as mice and ferrets before being proceeded to human clinical trials.

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Keywords: influenza A, A/H1N1 2009, hemagglutinin (HA), matrix 2 (M2) protein, ScFv, phage displayed-HuScFv library

Venomic Proteome of the King Cobra, *Ophiophagus hannah*

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Introduction: Venomous snakebite is an important public health problem in many tropical and subtropical countries. In Thailand, King cobra (*Ophiophagus hannah*) causes the most serious bites with the highest mortality.
Objective: To reveal proteome of the *O. hannah* holovenom.
Materials & Methods: Protein components of the *O. hannah* holovenom were separated by one dimensional gel electrophoresis. The separated protein components were then subjected to electrospray ionization liquid chromatography/ tandem mass spectrometry (ESI-LC/MS-MS) and orthologous protein identification.
Results: There were 45 orthologous proteins of the database matched with the peptide sequences derived from individual venom components. They were classified into 23 different groups according to functions and activities: zinc metalloproteinase, neurotoxins, phosphodiesterase, serine proteinase inhibitor, insulin-like growth factor I-like, hypothetical protein, phospholipases, metalloproteinase precursor, ankyrin repeat domain-containing protein, unnamed protein product, L-amino-acid oxidase, alpha- and beta- fibrinogenases, granzyme K-like, cobra venom factor, transmembrane protease, serine protease, hepatocyte growth factor activator, cardiotoxin, complement-depleting factor, opharin precursor, cysteine-rich secretory protein, Thai cobrin, weak toxin and muscarinic toxin.
Conclusion: This is the first report on *O. hannah* venomic proteome. The data should be useful for future design and production of the therapeutic antivenin and snake bite management.

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Keywords: *Ophiophagus hannah*, proteome, ESI-LC/MS-MS

Allergenome of House Dust Mite, *Dermatophagoides pteronyssinus*, among Atopic Thais

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Introduction: House dust mites (HDM), *Dermatophagoides pteronyssinus* (*Dp*) and *D. farinae* (*Df*) are major sources of indoor allergens causing type-1 allergic diseases with increasing incidence worldwide. Although extensive research on dust mite allergens has been carried out, the molecular characteristics of their allergenic components remain to be investigated further. Recently, advanced and complex system sciences, including various omics have been applied for revelation of genome, transciptome, proteome and metabolome of many organisms.
Objective: It was the aim of this study to reveal and characterize the proteome and allergenome of the *D. pteronyssinus*.
Materials & Methods: High-resolution two-dimensional gel electrophoresis (2-DE)-based-proteomics, 2DE-IgE immunoblotting, and database search were used. Whole body extract of cultured *Dp* with ~99% purity was subjected to a nonlinear pH 3-10 first dimensional electrophoresis followed by SDS-PAGE in 12% polyacrylamide gel. 2DE-IgE immunoblotting was carried out using individual sera of *Dp* allergic Thai patients (with ethical approval and informed consent). The IgE-reactive proteins were excised from the 2DE-gel and analyzed by matching the peptide mass maps generated after mass spectrometry with the protein database.
Results: Forty-six IgE-binding spots of *D. pteronyssinus* (*Dp*) were subjected to LC-MS/MS for protein identification. Many spots were major and known allergens including Der p 1, Der p 2, Der p 10, and Der p 13. Nevertheless, novel allergens were discovered in this study: myosin heavy chain and ferritin protein. Moreover, there were IgE reactive spots which could not be characterized due to limitation of the database. They may be regarded putatively as the *Dp* novel allergens.
Conclusion: Repertoire of *Dp* proteins (proteome) as well as the allergenic components (allergenome) were revealed readily by the high-resolution 2-DE technique combined with IgE-immunodetection. New allergens of *Dp* that reacted to the IgE in Thai patients' sera were discovered. Further characterization of these allergens should enable better understanding of their biological roles and degrees of allergenicity which should be useful information for a "tailor made allergen specific immunotherapy" of the house dust mite allergy.

Acknowledgements: The work was supported by the National Research University (NRU) project of the Office of the Commission on Higher education (CHE) and the Thailand Research Fund through DPG5380001 grant. Nitat Sookrung and Nitaya Indrawattana are TRF new scholars (mrg2554 and mrg2555, respectively).

Keywords: house dust mite allergen, 2-dimensional electrophoresis, *D. pteronyssinus*, IgE-immunodetection

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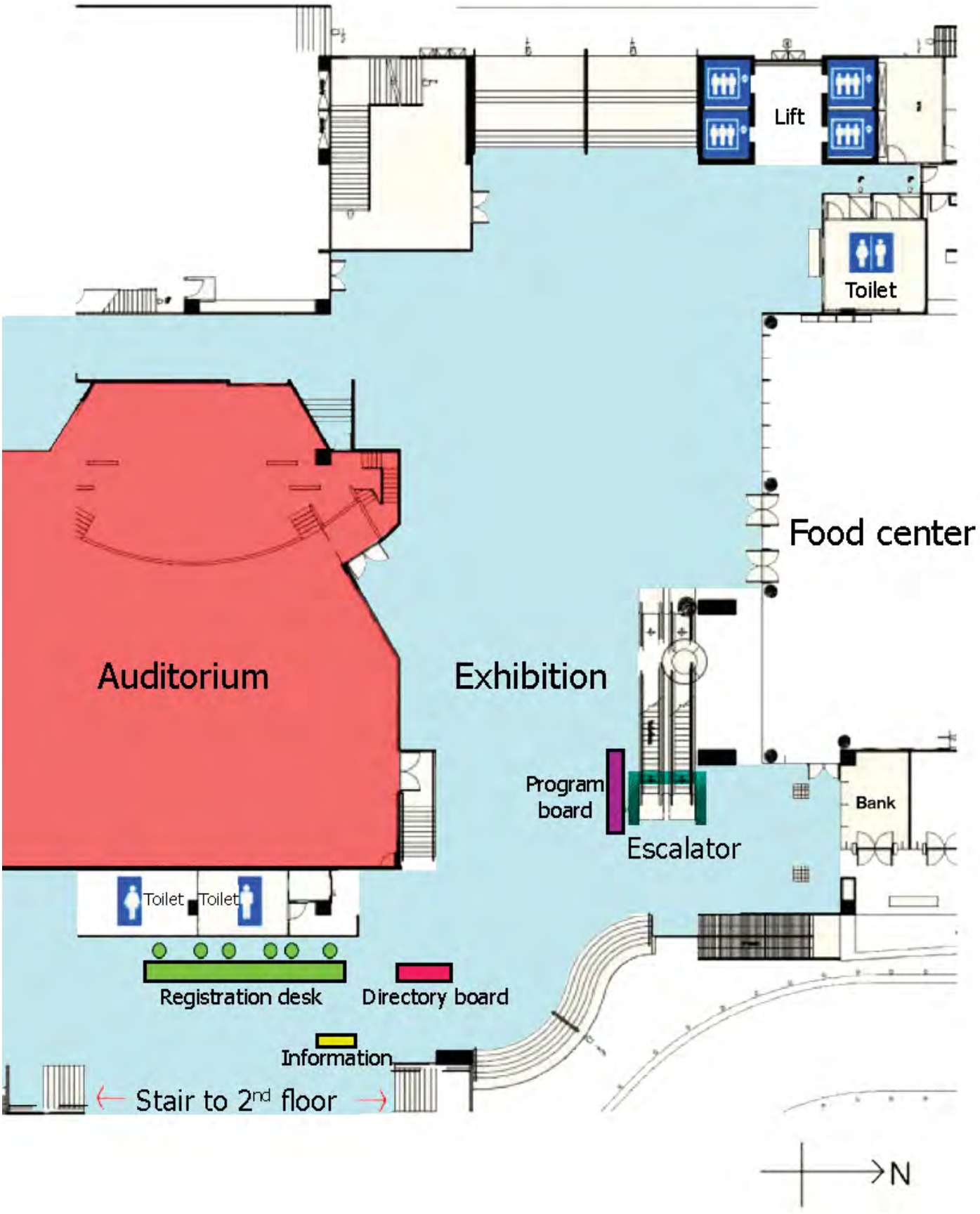
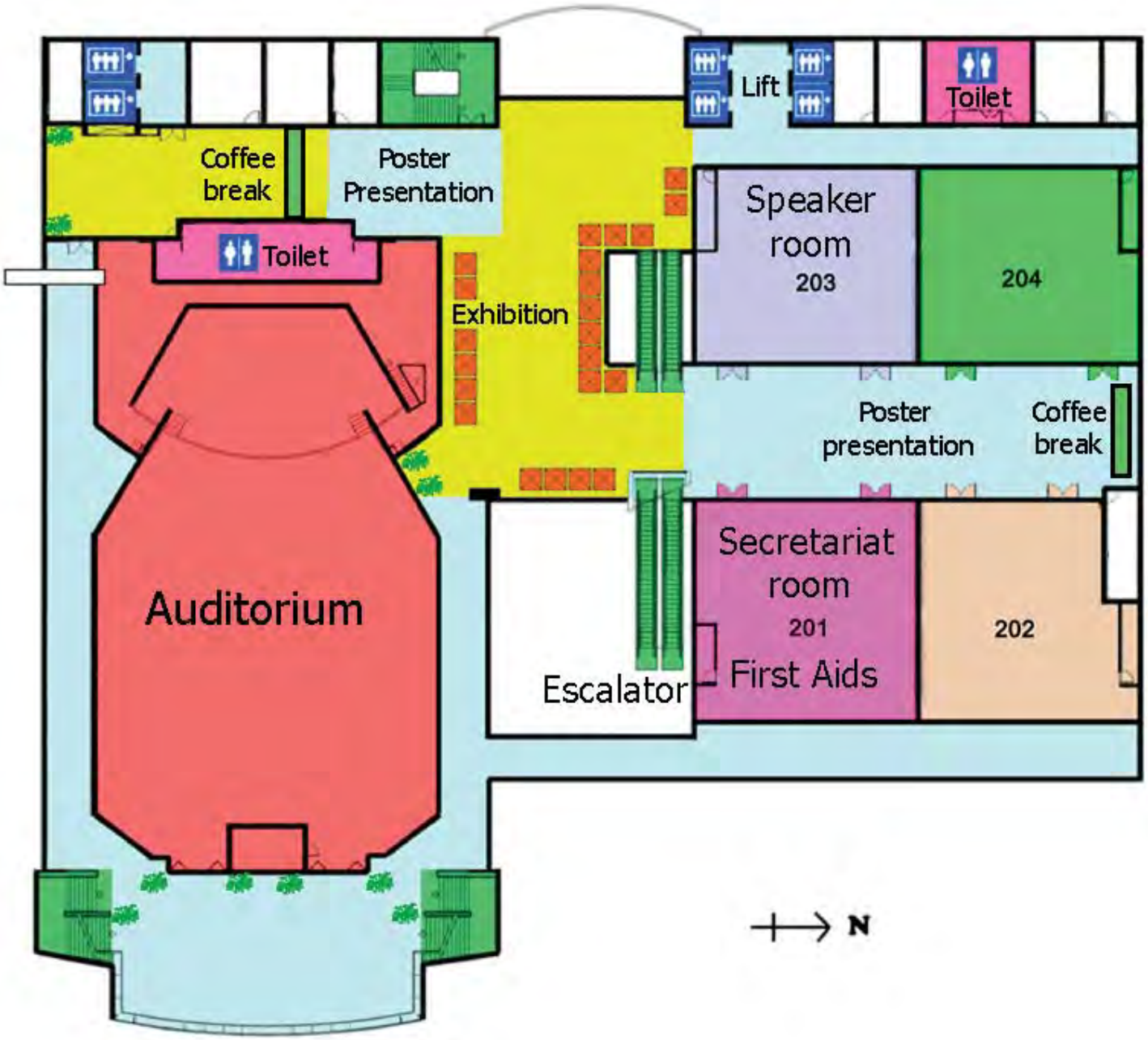
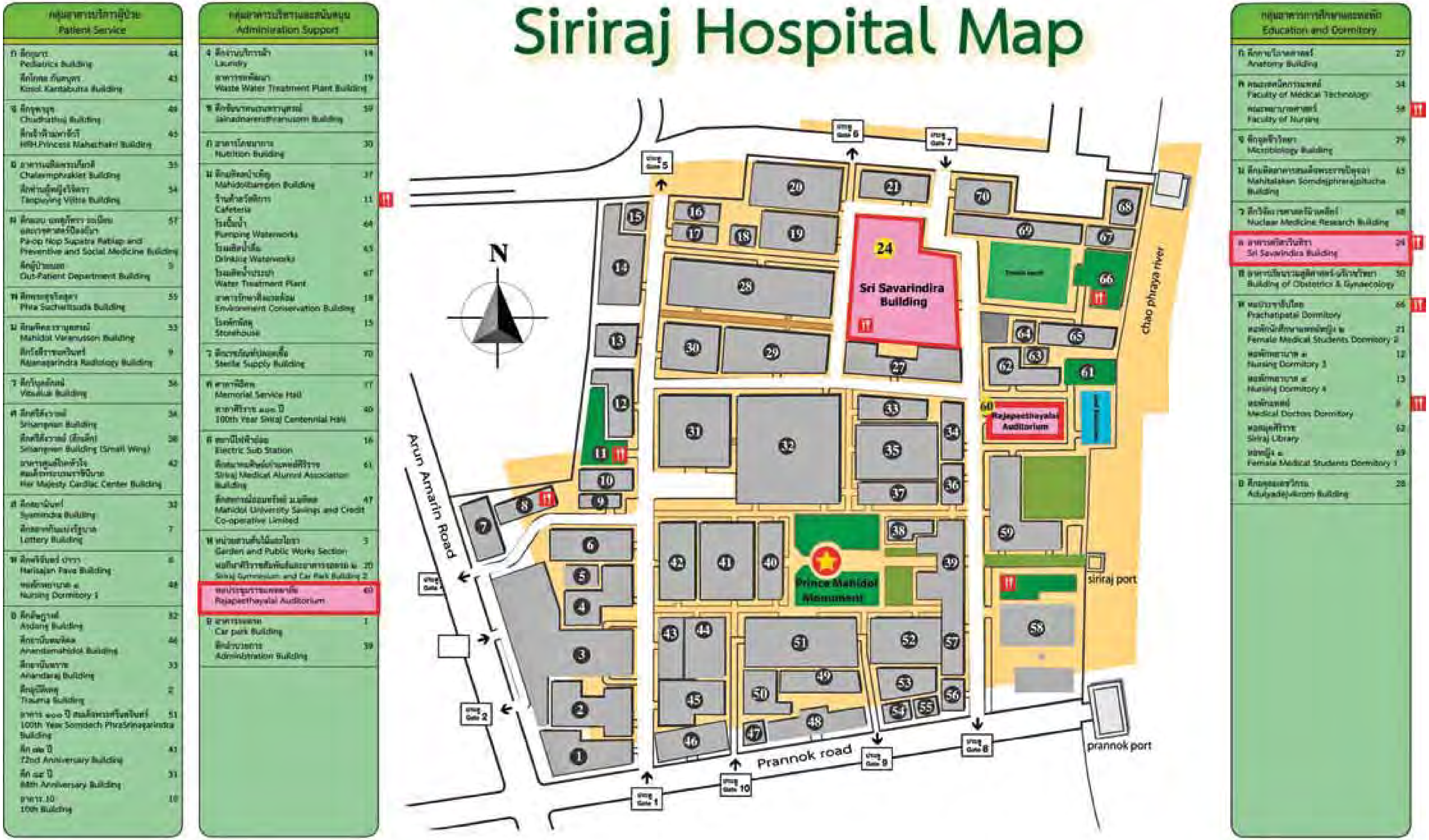


1st Floor: Srisavarindira Building



2nd Floor: Srisavarindira Building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December 19-22, 2012

Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

Addendum

Indolequinone Derivatives Inhibit TNFα-induced NF-κB Activation in Human Leukemia *via* Inhibition of NF-κB-DNA Binding Activity

Orlikova B.[1], Ross D.[2], Dicato M.[1], Diederich M.[1]

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Introduction: Indolequinones have been shown to act as potent antitumor agents. So far, two mechanistic targets were identified, underlining their properties: NADH quinone oxidoreductase and thioredoxin reductase. Nevertheless, there are indications pointing out that there should be additional mechanisms implicated in order to elucidate their total growth inhibitory effects on cancer cells. In the present study, we identified NF-κB as a novel molecular target responsible for their final antitumor effect.
Objective: We investigated how indolequinones interfere with the classical pathway of pro-inflammatory signaling through the TNFα-induced NF-κB pathway.
Materials & Methods: We examined the inhibitory effects of indolequinones on TNFα-induced NF-κB activation on transiently transfected cells using luciferase assays. To assess the percentage of metabolically active cells we applied The CellTiter-Glo® Assay. Western blotting was performed to analyze nuclear translocation of NF-κB subunits. We assessed indolequinones effects on NF-κB-DNA binding activity by EMSA. Transient transfection with ICAM-1 and quantitative real-time-PCR were performed to analyze ICAM-1 gene transcription and mRNA levels.
Results: Indolequinones inhibited dose-dependently TNFα-induced NF-κB pathway in K562 cells and expression of the NF-κB target gene ICAM-1 was down-regulated. Western blot analysis revealed that indolequinones are notable to restrain IB degradation and prevent p50/p65 nuclear translocation. Of importance, indolequinones inhibited cell viability in all tested leukemia cell lines but did not affect healthy cells.
Conclusion: Our study revealed for the first time that indolequinones act as potent inhibitors of the TNFα-induced NF-κB pathway. Moreover, preferential targeting of cancer cells by indolequinones makes them promising safe drug candidates in cancer treatment.

Keywords: indolequinones, inflammation, cancer, NF-κB pathway

2012
The 4th ASIAHORCs Joint Symposium

November 11th-14th, 2012, Lotte Resort, Buyeo

Organized by

National Research Foundation of Korea(NRF)
Infection Signaling Network Research Center, Chungnam National University
Biomedical Professional Education Initiative in Daedeok R&D complex,
Brain Korea 21, Chungnam National University School of Medicine

Schedule of the 4th ASIAHORCs Joint Symposium

Date and Avene: **November 11th-14th, 2012, Lotte Buyeo Resort, Buyeo, S. Korea**

Date		Time	Topics
November 11th (Sun)			
	PM	12:00 - 21:00	Participants arrival and Registration
	PM	**18:00 - 21:00**	**Wecome Reception (*Dalsol* HALL, Lotte Buyeo Resort)**
November 12th (Mon)			**SABI Hall, 1st floor in Lotte Resort**
		08:30 - 08:45	opening ceremony
	Sessions 1: *Immunology of Vaccination I* **Chair, Kiat Ruxrungtham (Chulalongkorn University)**		
		08:45 - 09:05	**Chao Qiu**, Boosting heterosubtypic neutralization antibodies in recipients of 2009 pandemic H1N1 influenza vaccine
		09:05 - 09:25	**Marimuthu MakeshKumar**, A phase 1 study to evaluate the safety and immunogenicity of a recombinant HIV Type 1 subtype C-modified vaccinia ankara virus vaccine candidate in indian volunteers
		09:25 - 09:50	**Tjandrawati Mozef**, Inhibition of platelet aggregation by some flavonoid isolated From the leaves of sukun, *Artocarpus Altilis* (Parkinson) Fosberg
	AM	**09:50 - 10:10**	**Coffee Break**
	Sessions 2: *Immunology of Vaccination II* **Chair, Jianqing Xu (Fudan University)**		
		10:10 - 10:35	**Jaime C. Montoya**, A randomized and controlled antigen and adjuvant dose-ranging phase II study of the M72/AS01 candidate tuberculosis vaccine in healthy PPD-positive adults
		10:35 - 11:00	**Vidya Arankalle**, The basis for development of vaccines for hepatitis E and *Chikungunya*
		11:00 - 11:25	**Kiat Ruxrungtham**, DNA vaccine development and strategies to improve immunogenicity at chulaVRC, Thailand
	AM	**11:25 - 12:30**	**Lunch (1st floor restaurant at the Lotte Resort)**
	Plenary Lecture **Chair, Jeong-Kyu Park (Chungnam National University)**		
	PM	12:30 - 13:10	**Bok Luel Lee,** Determination of molecular mechanisms of pathogen recognition and signaling pathway of host innate immune responses

Sessions 3: *Host-pathogen Interaction* **Chair, Kiyoshi Takeda (Osaka University)**		
	13:10 - 13:30	**Don-Kyu Kim**, The role of ERRgamma in host and pathogen interaction
	13:30 – 13:55	**Jianqing Xu**, Sequential priming and boosting with heterologous HIV immunogens predominantly stimulated cross-reactive effective memory T-cell immunity against different clades in non-human chinese *Rhesus Macaques*
	13:55 - 14:15	**Satoshi Koga**, The role of natural helper cells in *Nippostrongylus brasiliensis* infection
	14:15 - 14:40	**Eun-Kyeong Jo**, Innate immunity and autophagy in Mycobacterial Infection
PM	**14:40 - 15:00**	**Coffee Break**
Keynote Speech **Chair, Eun-Kyeong Jo (Chungnam National University)**		
	15:00 - 15:35	**Kiyoshi Takeda**, Regulation of intestinal inflammation by innate immunity
Sessions 4: *Host Immune Response Against Pathogens* **Chair, Jaime C. Montoya (Philippines University)**		
	15:35 - 15:55	**Miwa Sasai**, Adaptor protein 3 is required for type-I interferon production by toll-like receptor 9
	15:55 - 16:15	**Sunchai Payungporn**, Analysis of human microRNAs targeting influenza a viruses (subtype H1N1, H5N1 and H3N2)
	16:15 - 16:35	**Hiroki Kato**, RIG-I like receptors (RLRs) mediate antiviral innate immunity
	16:35 - 16:55	**Prafullakumar Tailor**, Interferon regulatory factor 8 plays a central role in CD8α+ dendritic cell development
	16:55 - 17:15	**Liangzhu Li**, Minocycline down-regulates topical mucosal inflammation during the application of microbicide candidates
	17:15 - 18:00	**Announce & Break Time**
Evening	**18:00 - 18:30**	**Gayagum Ochestra at the SABI HALL in the Lotte Resort**
	18:30 - 21:00	**Banquet at the SABI HALL in the Lotte Resort**
November 13th (Tue)		**SABI Hall, 1st floor in Lotte Resort**
Sessions 5: *Clinical Epidemiology* **Chair, Lei Gao (Chinese Academy of Medical Sciences)**		
	08:30 - 08:50	**Nitaya Indrawattana**, *Staphylococcus aureus* clinical isolates in Thailand: Antibiotic susceptibility and molecular characteristics

	08:50 - 09:10	**Parthasarthy Mohanty**, A study on transmission of environmental *Mycobacterium leprae* strains among leprosy patients
	09:10 - 09:30	**Li-Yen Chang**, Research on tropical infectious diseases at the tropical infectious diseases research and education centre (TIDREC), University of Malaya
	09:30 - 09:55	**Marie Dione P. Sacdalan**, Clinico-pathologic profile of patients with lower gastrointestinal tuberculosis in a tertiary hospital
Sessions 6: *Healthcare Epidemiology & Genetics* **Chair, Mun Yik Fong (Malaya University)**		
	09:55 - 10:15	**Nitat Sookrung**, Venomics and allergenomics of paper wasp, *Vespa affinis*, among Thais
	10:15 - 10:35	**Edsel Maurice T. Salvaña**, A Web-based interactive genome library for surveillance, detection, characterization and drug resistance monitoring of influenza virus infection in the Philippines
	10:35 - 10:55	**Adi Santoso**, Expression of modified glycosylation sites of human erythropoietin in mammalian cells
	10:55 - 11:20	**Lei Gao**, Genetic polymorphisms of toll-like receptor 9 and interferon-gamma is associated with the development of pulmonary tuberculosis in a chinese population
Sessions 7: *Recent studies of Infectious Diseases* **Chair, Vidya Arankalle (National Institute of Virology)**		
	11:20 - 11:35	**Jong-Seok Kim**, *Mycobacterium avium* subsp. *paratuberculsois* and *M. abscessus*: Emerging pathogenic mycobacteria as representatives of slowly growing and rapidly growing nontuberculous mycobacteria
	11:35 - 11:55	**Mun Yik Fong**, Molecular research on *Plasmodium knowlesi*, an emerging zoonotic malaria parasite
	11:55 - 12:10	**Chul-Su Yang**, Crossing the rubicon : New roads lead to host defense
	12:10 - 12:30	**Donna May D. Papa**, Alternative to antibiotics: Efficacy of bacteriophage therapy against burn wound infection in mice
	12:30 - 12:45	**Jae-Min Yuk**, The orphan nuclear receptor SHP acts as a negative regulator in toll-like receptor-triggered inflammatory signaling
PM	**12:45 - 13:40**	**Lunch (1st floor restaurant at the Lotte Resort)**
PM	**13:40 - 18:00**	**Half Day TOUR (For ASIAHORCs participants)**
PM	**18:00 -**	**Dinner**
November 14th (Wed)		Participants depart for home

Staphylococcus aureus Clinical Isolates in Thailand: Antibiotic Susceptibility and Molecular Characteristics

Nitaya Indrawattana[1], Orawan Sungkhachat[1], Nitat Sookrung[2], Manas Chongsa-nguan[1], Anchalee Tungtongchitr[3], Supayang P. Voravuthikunchai[4], Thida Kong-ngoen[1], Hisao Kurazono[5] and Wanpen Chaicumpa[3]

[1]Department of Microbiology and Immunology, Faculty of Tropical Medicine, [2]Department of Research and Development, [3]Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand; [4]Natural Products Research Center and Department of Microbiology, Faculty of Science, Prince of Songkhla University, Songkhla, Thailand; [5]Department of Applied Veterinary Medicine and Public Health, Obihiro University of Agriculture and Veterinary Medicine, Hokkaido, Japan

Staphylococcus aureus, a gram-positive coccal bacterium, is an important human pathogen causing a wide variety of diseases: food poisoning, local blistering skin disease (bullous impetigo)/generalized scalded skin syndrome, pneumonitis, sepsis and toxic shock syndrome. The bacterium produces a multitude of virulent factors including adhesins, toxic proteins/enzymes and exotoxins, which encoded mainly by mobile genetic elements. β-lactam antibiotics are drugs of choice for treatment of *S. aureus* infections. However, drug resistant strains emerge continuously, especially the methicillin-resistant *S. aureus* (MRSA) which raised concerns of microbiologists and clinicians. Periodic monitoring of *S. aureus* isolates in a locality for their antibiotic susceptibility and molecular characteristics are imperative. In this study, antibiograms, prevalence of toxin genes coding for enterotoxins (*sea-see*, seg-*ser*, *seu*), toxic shock syndrome toxin-1 (*sef* or *tsst-1*), and exfoliative toxins (*eta*, *etb* and *etd*), as well as molecular characteristics (PFGE and types of accessory gene regulator, *agr*) of 92 *S. aureus* isolates of three hospitals in Thailand were investigated. Based on the antimicrobial susceptibility, they were classified arbitrarily into 10 different drug groups. Groups 1-7 (56 isolates) were MRSA; groups 8-10 (36 isolates) were MSSA. For prevalence of toxin genes (*se* and *ext*): 94.57% carried two or more genes, 4.35% carried one gene (*seq*) and 1 isolate (1.08%) did not have any gene. No isolate carried the *etb* and *tsst-1* (*sef*). The *etx* genes, either *eta* or *etd*, were found in only 6/92 isolates. For the other enterotoxin genes, 20.5% of the isolates were PCR positive for *sek* and *seq*, 19.5% for *sea*, *sek* and *seq*, 5.4% for *sea*, *seg*, *sei*, *sek*, *sen*, *seo* and

seq, 5.4% for *sea*, *seg*, *sei*, *sek*, *sem*, *sen*, *seo* and *seq*, and 3.2% for *sea*, *seb*, *seg*, *sei*, *sek*, *sem*, *sen*, *seo*, *seq*; 1% of the isolates did not carry any *se* gene. The 92 isolates revealed 23 different PFGE types; 58.70, 31.52, 6.52 and 3.26% of them belonged to *agr* groups 1-4, respectively. The results of this study provide the first data set on genotypic and phenotypic characteristics of *S. aureus* isolates in Thailand which should be useful for future active surveillance that aimed to control anti-microbial resistant bacteria.

Keyword: *Staphylococcus aureus*, staphylococcal enterotoxins, MRSA, *agr*, PFGE

Venomics and allergenomics of paper wasp, *Vespa affinis*, among Thais

Nitat Sookrung[1], Siriporn Wongdindam[2], Nitaya Indrawattana[3], Anchalee Tungtongchitr[4], Wiparat Manuyakorn[5], Kovit Pattanapanyasat[1] and Wanpen Chaicumpa[4]

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[3]Department of Microbiology and Immunology, Faculty of Tropical Medicine, Mahidol University, Bangkok, 10400, Thailand, [5]Department of Pediatrics, Ramathibodi Hospital, Faculty of Medicine, Mahidol University, Bangkok, 10400, Thailand.

Wasp venom allergy which is an IgE-mediated (type-1) hypersensitivity. In Thailand, the predominant wasp species is *Vespa affinis*. The most frequent wasp sting clinical patterns are large local reaction and some patient may develop severe systemic reaction leading to anaphylactic shock, acute renal failure and eventually death. Information on wasp venom allergy, including the venom nature, molecular structure, biological and physiological functions and their role as allergens among patients, especially Thais, are scarce. In this study we separated *Vespa affinis* 94 venom components from venom sac by two dimensional gel electrophoresis. Sixty six protein spots were identified by LC-MS/MS on their amino acid sequences and 28 protein spots no significant match to proteins in database. Individual components of the venom were reacted with IgE from *Vespa affinis* allergic Thai patients for allergenome identification. IgE from most patients can bind four major allergens, Phospholipase A1 (92.3%), GB19860 transcription protein (76.9%), Enolase (61.5%), venom allergen 5 (61.5%). Some patients produce specific IgE to minor allergen are hyaluronidase (46.1%) and arginine kinase-like protein (46.1%). The results obtained from this research will give insight into the venomics of *Vespa affinis* and the allergenic components. This information should lead to the way of proper diagnosis, screening, monitoring and treatment of the patients with wasp venom allergy in Thailand.

Keywords: *Vespa affinis*, venomics, allergenomics, Two dimensional gel electrophoresis, phospholipase A 1