

Abstract

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Project Title:

Tumor specific glycolytic enzymes promote progression of cholangiocarcinoma

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1. Abstract

Cancer cells differ from normal cells in their metabolic properties. In aerobic condition, normal cells mostly rely on mitochondrial oxidative phosphorylation to produce energy. In contrast, cancer cells, although in aerobic atmosphere, depend mostly on glycolysis, the aerobic breakdown of glucose into ATP. This altered energy dependency is known as the “Warburg effect”. A better understanding of the unique metabolism of cancer cells will help to design of more effective drugs and biomarkers, which will be greatly impact for the effective treatment and diagnosis of cancer patients. However, the metabolic reprogramming of cholangiocarcinoma (CCA) has not yet been investigated. This study is aimed to verify the expression profile of tumor specific glycolytic enzymes in CCA. The consequence of these tumor specific glycolytic proteins in association with CCA progression will be explores. Based on the high-throughput screening database of CCA (Serial analysis of gene expression and cDNA microarray), four tumor specific glycolytic proteins including glucose transporter 1 (GLUT1), hexokinase 2 (HK2), pyruvate kinase M2 (PKM2), lactate dehydrogenase A (LDHA), were selected. The expression of GLUT1, HK2, PKM2 and LDHA were rarely expressed in normal bile duct epithelium, but was expressed in hyperplastic/dysplastic and CCA. The observation was confirmed in the Ov associated

hamster model. In addition, higher GLUT1 and LDHA expression were associated with shorter survival of CCA patients. Suppression of GLUT1 expression/action using siRNA/specific inhibitor significantly decreased cell proliferation, migration and invasion of CCA cell lines. Similar results were obtained in HK2 and PKM2 suppressed CCA cell lines. These results indicate the association of tumor specific glycolytic proteins in cholangiocarcinogenesis/progression and suggest the possibility of tumor specific glycolytic proteins as a therapeutic target for CCA.

2. Executive summary

Cholangiocarcinoma (CCA), an aggressive and lethal cancer, arises from biliary epithelium. The global incidence of CCA is low compared with that of hepatoma, although the rates of mortality and incidence of CCA have been noted increasingly worldwide. Because CCA is difficult to diagnose at an early stage, almost all patients with CCA present with advanced, incurable disease. Even in patients who have undergone complete surgical resection, the recurrence rate remains quite high; consequently, the low 5-year survival rate ranged from 0% to 40%. Therefore, novel molecular-targeted therapeutics and prognostic markers directed against this malignancy constitute an urgent need. Alteration of metabolism is one of the hallmarks of cancer cells. The best-known metabolic abnormality in cancer cells is the Warburg effect, which demonstrates an increased glycolysis even in the presence of oxygen which called “aerobic glycolysis”. Key tumor genes such as p53, c-myc and hypoxia-Inducible Factor-1 (HIF-1) are found to be the master regulators of aerobic glycolysis which controls the expression of tumor specific glycolytic enzymes and its related proteins such as glucose transporter 1 (GLUT1), hexokinase 2 (HK2), pyruvate kinase M2 (PKM2), lactate dehydrogenase A (LDHA), monocarboxylate transporter 1 (MCT1). Overexpression of GLUT1, HK2, PKM2, LDHA and MCT1 are associated with aggressive phenotypes of cancer cells including high proliferation rate, survival, metastasis as well as chemotherapy and radiotherapy resistances. Suppression of these tumor specific glycolytic enzyme expressions by siRNA or shRNA significantly decreased both the growth and migratory potential of several cancer cells. In recent years, there are increasing evidences pointing at interventions on tumor glycolysis as a novel strategy for diagnosis and selective anti-cancer therapies. Therefore, drugs resulting from this approach are supposed to be highly selective against cancer cells and, therefore, devoid of important undesirable side effects, since cancer cells display an exaggerated addiction to glycolysis, when compared to normal cells. Many of the key effectors of tumor specific glycolytic enzymes and its related proteins can be considered as promising targets for therapeutic intervention against cancer. These tumor specific glycolytic enzymes are overexpressed in invasive tumors and, therefore, offer a relatively safe therapeutic window for anticancer

agents that target them. Presently, there are several small molecules at the preclinical that are reported to act as metabolic modulators in cancer cells and some drug candidates are advanced to phase II/III clinical trials. At present, the alterations of expressed genes at molecular level in CCA have been obtained by different high-throughput techniques. Serial analysis of gene expression (SAGE) and cDNA microarray were used to identify differentially expressed genes in CCA (Andersen, Spee et al. 2012; Andersen and Thorgeirsson 2012; Sawanyawisuth, Wongkham et al. 2012; Sia, Hoshida et al. 2013). These high-throughput techniques revealed several up-regulated genes in CCA tissues including GLUT1, HK2, PKM2 and LDHA. However, roles of these glycolytic enzymes in CCA progression have not been clearly elucidated. In this study the expression and association of tumor glycolytic enzymes namely GLUT1, HK2, PKM2 and LDHA with CCA progression were explored in CCA cell lines and tissues. The overexpression of GLUT1, HK2, PKM2 and LDHA were found during carcinogenesis of CCA. The observation was confirmed in the Ov associated hamster CCA model. In addition, the overexpression of GLUT1 and LDHA were associated with shorter survival of CCA patients. Suppression of GLUT1 expression/action using siRNA/specific inhibitor significantly decreased cell proliferation, migration and invasion of CCA cell lines. Similar results were obtained in HK2 and PKM2 suppressed CCA cell lines. The information obtained from this study may fulfill the understanding of CCA progression and facilitate the improvement of diagnosis and targeted therapy which is finally benefited to CCA patients.

Not only the novel knowledge on CCA that was gained from this research project, two MSc students from Faculty of medicine, Khon Kaen University (Miss Unchalee Thonsri and Miss Ubonrat Thamrongwarangoon) were also graduated her master program under this research project. In addition, at least 2 international publications were produced and another 2 manuscripts will be submitted into international publication within this year.

3. Objective

3.1 To determine the expression profile of tumor specific glycolytic enzymes namely GLUT1, HK2, PKM2, LDHA in CCA cell lines and CCA patient tissues.

3.2 To investigate the association between expression levels of GLUT1, HK2, PKM2, LDHA and clinicopathological findings/survival of CCA patients.

3.3 To verify the significant roles of the particular glycolytic enzymes on proliferation, motility and apoptosis of CCA cell lines.

4. Research methodology

In this study, we hypothesize that CCA possesses aberrant tumor specific glycolytic enzymes GLUT1, HK2, PKM2 and LDHA which mediate tumor progression of CCA. The expression levels of tumor specific glycolytic enzymes i.e., GLUT1, HK2, PKM2 and LDHA, were determined in CCA patient tissues using immunohistochemistry technique. The significance of tumor specific glycolytic enzymes levels related to disease were explored. In addition, the association of tumor specific glycolytic enzymes and progression of CCA (proliferation, wound healing, migration, invasion, adhesion, etc.) were determined in the CCA cell lines. Small interfering RNA (siRNA)/specific inhibitor to candidate tumor specific glycolytic enzymes was used to suppress the expression/action. The experimental design for the proposed project is shown below.