รายงาน
ฉบับสมบูรณ์
ทุนพัฒนานักวิจัย รุ่นที่ 1
สัญญาเลขที่ RSA/19/2537

ชีววิทยาโมเลกุลการเกิดมะเร็งหลังโพรงจมูก

Molecular Biology of Nasopharyngeal Carcinogenesis

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ทุนพัฒนานักวิจัย รุ่นที่ 1

สัญญาเลขที่ RSA/19/2537

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Abstract

This study has explored molecular biology of nasopharyngeal carcinoma (NPC) and head & neck squamous cell carcinoma (HNSCC) developments. We succeeded in studying three major topics, genetic alterations in Epstein-Barr viral (EBV) associated NPC, EBV DNA in serum of patients with NPC, and telomerase activity in oral leukoplakia and HNSCC.

Genetic Alterations in EBV Associated NPC

Nasopharyngeal carcinoma is a subset of HNSCC with unique endemic distribution and aetiological co-factors. EBV has been revealed to be an important aetiological factor for most nasopharyngeal carcinomas. Nevertheless, additional genetic alterations may be involved in their development and progression. The aim of this study was to determine the likely chromosomal locations of tumour-suppressor genes related to EBV associated NPC. Fifty-six microsatellite polymorphic markers located on every autosomal arm were used to estimate the incidence of loss of heterozygosity (LOH) in 27 EBV associated NPC. High frequencies of allelic loss were observed on chromosome 3p (75.0%) and 9p (87.0%). Chromosome 9q, 11q, 13q and 14q displayed loss in over 50%, while chromosome 3q, 6p, 16q, 19q and 22q exhibited loss in 35-50%. Furthermore, several other chromosomal arms demonstrated allelic loss in 20-35%. Additionally, 1 of the 27 cases showed microsatellite instability at multiple loci. These finding provide evidence of multiple genetic alterations during cancer development and clues for further studies of tumor-suppressor genes in EBV associated NPC.

Since the incidence of LOH in NPC to amount to a frequency of loss on chromosome 3, 9, 11q, 13q and 14q exceeding 50%, there must be crucial tumor suppressor genes for NPC development located on these chromosomes. Detail mapping on these chromosomes would reveal these valuable informations. Previous studies and our data reveal three, one and three tumor supressor gene loci on chromsome 3, 9,and 11q respectively. Additional LOH mapping study was performed on chromosome 14q to define its minimal deletion regions. Forty tumors were selected for PCR-based deletion mapping using 16 microsatellite polymorphic markers spanning the long arm of this chromosome. LOH for at least one marker was observed in 29 (72.5%) tumors. Twenty two of these tumors displayed partial loss and provided an informative basis for detailed deletion mapping. Three minimal regions of loss were delineated. The first region was defined by markers D14S278 and D14S288 and the second was between D14S51 and telomere. These data confirmed two potential tumor suppressor gene loci at 14q12-13 and 14q32, respectively. Interestingly, the third region of loss was located on the T-cell receptor delta chain locus. This may reflect another tumor suppressor gene locus or may be a consequence of a specific genomic rearrangement of this region. In addition, these allelic losses occurred with high frequency in all tumor grades and stages and in all histological subtypes. These findings suggested that the genetic alteration of chromosome 14 is common and crucial during NPC development.

EBV DNA in Serum of Patients with NPC

This study evaluated EBV DNA in sera of 46 patients with NPC and 84 healthy individuals who previously been infected with EBV. Thirteen out of 46 NPC samples were positive for EBV DNA in their sera while all 84 normal controls were negative. In addition, EBV typing between primary tumors and sera showed identical results suggesting that serum EBV DNA represented tumor DNA. To evaluate the importance of the serum NPC DNA, clinical data and tumor phenotypes including age, sex, WHO type, EBV type, stage, tumor invasion, metastasis and apoptosis were correlated with serum EBV DNA and only apoptosis was found statistically significant. In conclusion, EBV DNA was detectable in the serum of some patients with NPC and represented tumor DNA. In addition, this finding was associated with tumor cell death and might have clinical implications in the future.

Telomerase Activity in Oral Leukoplakia and HNSCC.

The expression of telomerase, a ribonucleoprotein complex, is necessary to overcome cellular senescence, and it is associated with immortal cells and cancer. However, its role in precancerous lesions such as oral leukoplakias is less known. The purpose of this study is to investigate the presence of telomerase activity in oral leukoplakia and the relationship between the enzyme and multistep tumorigenesis. Telomerase activity was detectable in 14 of 16 HNSCCs and 10 of 26 oral leukoplakia tissues. We also showed that the expression of telomerase in the premalignant lesions was associated with phenotypic progression, the degree of dysplasia. These results indicate that telomerase is activated frequently during the late stage oral premalignancy and may play a crucial role in head and neck squamous cell carcinogenesis.

Note:

Genetic alterations in Epstein-Barr viral associated NPC is described in chapter 1 and 2.
EBV DNA in serum of patients with NPC is described in chapter 3.
Telomerase activity in oral leukoplakia and HNSCC is described in chapter 4.

บทคัดย่อ

การศึกษานี้ ศึกษาอณูชีววิทยาการพัฒนาของมะเร็งหลังโพรงจมูก และมะเร็งศรีษะและคอแบบสความัส เราสามารถสำเร็จการศึกษาใน 3 หัวข้อคือ การเปลี่ยนแปลงของพันธุกรรมในมะเร็งหลังโพรงจมูกที่ติดเชื้อเอ็ปสไตล์บาร์ไวรัส ดีเอ็นเอของเอ็ปสไตล์บาร์ไวรัสในซีลัมของผู้ป่วยมะเร็งหลังโพรงจมูก และการทำงานของเอ็นไซม์ทีโลเมอร์เรสในเนื้อเยื่อก่อนเกิดมะเร็งของช่องปากและมะเร็งศรีษะและคอแบบสความัส

การเปลี่ยนแปลงของพันธุกรรมในมะเร็งหลังโพรงจมูกที่ติดเชื้อเอ็ปสไตล์บาร์ไวรัส

มะเร็งหลังโพรงจมูกถูกจัดอยู่ในมะเร็งศรีษะและคอแบบสความัสที่มีลักษณะจำเพาะของการกระจายตัวและสาเหตุเอ็ปสไตล์บาร์ไวรัสถูกพบว่าเป็นสาเหตุที่สำคัญของมะเร็งหลังโพรงจมูกโดยส่วนใหญ่ อย่างไรก็ตามการกลายพันธ์ของเนื้อเยื่อน่าจะมีส่วนในการพัฒนาของมะเร็ง วัตถุประสงค์ของการศึกษานี้เพื่อที่จะวินิจฉัยตำแหน่งของโครโมโซมที่มียีนต้านมะเร็งที่เกี่ยวข้องกับมะเร็งหลังโพรงจมูกที่ติดเชื้อเอ็ปสไตล์บาร์ไวรัส ไมโครแซตเทิลไลท์ 56 อัน ที่พบกระจายอยู่ทุกแขนของออโตโซมถูกนำมาใช้เพื่อศึกษาความถี่ของการเกิดภาวะสูญเสียเฮทเทอร์โรไซโกท ในมะเร็งหลังโพรงจมูกที่ติดเชื้อเอ็ปสไตล์บาร์ไวรัส 27 ราย ความถี่ที่สูงของภาวะนี้ถูกพบในโครโทโซมที่ 3p (75.0%) และ 9p (87.0%) โครโมโซม 9q 11q 13q และ 14q พบภาวะสูญเสียมากกว่า 50% ในขณะที่โครโมโซม 3q 6p 16q 19q และ 22q แสดงภาวะสูญเสีย 35-50% นอกจากนี้แขนโครโมโซมอื่น ๆ บางโครโมโซม พบภาวะสูญเสียได้ 20-35% นอกจากนี้ 1 ราย ใน 27 ราย พบภาวะไม่เสถียรของไมโครแซตเทิลไลท์ในหลายตำแหน่ง การศึกษานี้แสดงถึงการกลายพันธุ์ในหลายตำแหน่งในระหว่างการพัฒนาของมะเร็งและเป็นข้อมูลสำหรับการศึกษาสำหรับยีนต้านมะเร็งในมะเร็งหลังโพรงจมูกที่ติดเชื้อเอ็ปสไตล์บาร์ไวรัส

เนื่องจากภาวะสูญเสียเฮทเทอร์โรไซโกทในมะเร็งหลังโพรงจมูกพบความถี่สูงที่โครโมโซม 3 9 11q 13q และ 14q มากกว่า 50% จึงน่าจะมียีนต้านมะเร็งที่สำคัญสำหรับการเจริญของมะเร็งหลังโพรงจมูกอยู่บนโครโมโซมต่าง ๆ เหล่านี้ การศึกษาแผนที่โครโมโซมอย่างละเอียดจะสามารถให้ข้อมูลเหล่านี้ได้ การศึกษาที่ผ่านมาพบมียีนต้านมะเร็งอยู่ 3 1 และ 3 ตำแหน่งบนโครโมโซม 3 9 และ 11q ตามลำดับ เราได้ทำการศึกษาการทำแผนที่ภาวะเฮทเทอร์โรไซโกทบนโครโมโซม 14q เพื่อค้นหาตำแหน่งการขาดหายที่สั้นที่สุด มะเร็ง 40 ราย ถูกเลือกมาศึกษาแผนที่การขาดหายด้วยพีซีอาร์โดยใช้ไมโครแซตเทิลไลท์ 16 อัน บนแขนยาวของโครโมโซมนี้ ภาวะสูญเสียอย่างน้อย 1 ตำแหน่ง ถูกสังเกตุในมะเร็ง 29 ราย (72.5%) มะเร็ง 22 ราย แสดงการสูญเสียของบางตำแหน่งและมีประโยชน์สำหรับให้ข้อมูลอย่างละเอียดของแผนที่การขาดหาย การขาดหายพบว่ามีตำแหน่งที่สำคัญ 3 ตำแหน่ง ตำแหน่งแรกพบอยู่ระหว่าง D14S278 และ D14S288 และตำแหน่งที่สอง พบอยู่ระหว่าง D14S51 และ ทีโลเมียร์ ข้อมูลนี้ยืนยันว่ามียีนต้านมะเร็งที่ 14q12-13 และ 14q32 ตามลำดับ ที่น่าสนใจคือ การสูญเสียในตำแหน่งที่ 3 ถูกพบอยู่ในตำแหน่งของ T-cell receptor delta chain (TCRD) ซึ่งอาจจะหมายถึงตำแหน่งของยีนต้านมะเร็งหรืออาจจะเป็นผลของการเปลี่ยนแปลงของจีโนมในตำแหน่งนี้ นอกจากนี้ภาวะสูญเสียพบใน

ความถี่สูงในมะเร็งทุกระยะทุกชนิด การศึกษานี้ยืนยันว่าการกลายพันธุ์ของโครโมโซมที่ 14 พบบ่อยและมีความสำคัญต่อการพัฒนาของมะเร็งหลังโพรงจมูก

ดีเอ็นเอของเอปสไตล์บาร์ไวรัสในซีลัมของผู้ป่วยมะเร็งหลังโพรงจมูก

การศึกษานี้ศึกษาดีเอ็นเอของเอ็ปสไตล์บาร์ไวรัสในซีรั่มของผู้ป่วยมะเร็งหลังโพรงจมูก 46 ราย และคนปกติที่ถูกพบว่าเคยติดเชื้อเอ็ปสไตล์บาร์ไวรัส 84 ราย ผู้ป่วย 13 รายจาก 46 ราย พบมีดีเอ็นเอของเอ็ปสไตล์บาร์ไวรัสในซีรัมขณะที่ซีรั่มของคนปกติ 84 ราย ให้ผลลบ นอกจากนี้ การจำแนกเชื้อเอ็ปสไตล์บาร์ไวรัสจากก้อนเนื้อและซีรั่มให้ผลเหมือนกันแสดงว่าดีเอ็นเอของเชื้อเอ็ปสไตล์บาร์ไวรัสในซีรั่มเป็นดีเอ็นเอจากก้อนมะเร็ง เพื่อศึกษาถึงความสำคัญของดีเอ็นเอของมะเร็งหลังโพรงจมูกในซีรั่ม ข้อมูลทางคลีนิค ลักษณะของมะเร็ง อายุ เพศ ชนิด WHO ชนิดเชื้อเอ็ปสไตล์บาร์ไวรัส ระยะ การเบียดแทรกจากก้อนเนื้อ การกระจายตัว และ โปรแกรมการตายถูกนำมาเปรียบเทียบกับดีเอ็นเอของเอ็ปสไตล์บาร์ไวรัสในซีรั่มและพบเฉพาะโปรแกรมการตายมีนัยสำคัญทางสถิติ โดยสรุปดีเอ็นเอของเอ็ปสไตล์บาร์ไวรัสสามารถตรวจพบในซีรั่มของผู้ป่วยโรคมะเร็งหลังโพรงจมูกและแสดงถึงดีเอ็นเอของเนื้อมะเร็ง นอกจากนี้การค้นพบนี้ยังมีความสัมพันธ์กับการตายของเนื้องอกและอาจมีความสัมพันธ์ต่อการนำมาใช้ทางคลินิคในอนาคต

การทำงานของเอ็นไซม์ทีโลเมอร์เรสในเนื้อเยื่อก่อนเกิดมะเร็งของช่องปากและมะเร็งศรีษะและคอแบบสความัส

การแสดงออกของเอ็นไซม์ทีโลเมอร์เรสซึ่งเป็นไรโบนิวคลีโอโปรตีนมีความสำคัญต่อการเอาชนะการแก่ของเซลล์และมีความสัมพันธ์ต่อภาวะไม่ตายของเซลล์และมะเร็ง อย่างไรก็ดีบทบาทของเอ็นไซม์นี้ในเนื้อเยื่อก่อนเกิดมะเร็งเช่นเนื้อเยื่อก่อนเกิดมะเร็งของช่องปากยังเป็นที่รู้น้อย วัตถุประสงค์ของการศึกษานี้เพื่อจะสำรวจการทำงานของเอ็นไซม์ทีโลเมอร์เรสในเนื้อเยื่อก่อนเกิดมะเร็งในช่องปากและความสัมพันธ์ระหว่างเอ็นไซม์นี้กับการเกิดมะเร็งแบบหลายขั้นตอน การทำงานของเอ็นไซม์ทีโลเมอร์เรสถูกพบในมะเร็งของศรีษะและคอแบบสความัสใน 14 ราย จาก 16 ราย และพบในเนื้อเยื่อก่อนเกิดมะเร็งในช่องปาก 10 ราย จาก 26 ราย เรายังพบอีกว่าการแสดงออกของเอ็นไซม์ทีโลเมอร์เรสมีความสัมพันธ์กับลักษณะการพัฒนาทางรูปร่างของเนื้อเยื่อก่อนเกิดมะเร็ง เช่น ระดับของการเสียรูปร่างในระดับกล้องจุลทรรศน์ ผลการตรวจพบนี้แสดงว่าการทำงานของเอ็นไซม์ทีโลเมอร์เรสถูกกระตุ้นอย่างบ่อยในระยะท้ายของเนื้อเยื่อก่อนเกิดมะเร็งในช่องปากและน่าจะมีความสำคัญต่อการเกิดมะเร็งของศรีษะและคอแบบสความัส

Chapter 1

Genomic Alterations in Nasopharyngeal Carcinoma: Loss of Heterozygosity and Epstein-Barr Virus Infection

This results presented in this chapter have been taken, in part, from a published manuscript

- **Mutirangura A**, Tanunyutthawongese C, Kerekhanjanarong V, Sriuranpong V, Yenrudi S, Supiyaphun P and Voravud N. Genomic alterations in nasopharyngeal carcinoma: loss of heterozygosity and Epstein-Barr virus infection.
  British Journal of Cancer; 1997;76(6), 770-776.
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- Tanunyutthawongese C, Kerekhanjanarong V, Sriuranpong V, Pornthanakasem W, Yenrudi S, Changchup B, Supiyaphun P, Voravud N and **Mutirangura A**. Microsatellite instability in Epstein-Barr-virus associated nasopharyngeal carcinoma.
  J Med Assoc Thai; 1996; 79 (Suppl 1), 71-77.

Introduction

Nasopharyngeal carcinoma (NPC) is a subset of head and neck squamous cell cancers (HNSCC) with unique endemic distribution and etiologic co-factors (1). Although NPC is rare among Caucasians in Europe and North America, it is one of the most common cancers in Southern China and among Eskimos in Arctic regions where it has an incidence of 20 to 50 per 100,000 males. An intermediate incidence is noted in Southeast Asia (2). While HNSCC is closely associated with exposure to tobacco and alcohol, Epstein-Barr-Virus (EBV) appears to be an important etiologic factor for most NPC (3).

Loss of function of tumor suppressor genes has been implicated as being essential for solid tumor development and related to chromosomal rearrangement regarding the loss of normal chromosomes or segments (4, 5). Various studies in NPC reported frequent allelic loss on chromosome 3p, 9p and 11q and homozygous deletion or hypermethylation of the p16 gene (6-11). The aim of this study is to investigate whether other tumor suppressor genes are also involved in NPC development by analysing the loss of heterozygosity (LOH) on every autosomal arm. Interestingly, allelotyping of HNSCC have been well characterized (12-14). Allelic loss on chromosome 3p, 9p and 11q are also frequent events in HNSCC. In addition, frequent LOH was observed on other chromosomes, e.g., 6p, 8, 13q, 14q, 17p, 18q and 19q (12-14). It would be of great interest and importance to elucidate whether the genetic events in Epstein-Barr-Virus-associated NPC are similar or distinct from HNSCC.

Materials and Methods

Tissues and DNA Extraction.

Primary NPC tissues were collected from 27 patients prior to treatment at Chulalongkorn University Hospital. The tissues were divided into two pieces. The first part was sent for routine histological examination. The second part was immediately stored in liquid nitrogen until further use. All the tumors were histologically ascertained to be undifferentiated NPC according to the WHO classification. The 27 tumors included stages ranging from II to IV. Blood samples obtained by venipuncture from the same patients were

used as constitutional controls. DNA was extracted from the tumor tissues and blood leukocytes by methods previously described (15).

EBV detection and typing by PCR

For the detection and typing of EBV DNA in the tumor tissues, three previously described PCR protocols were used with some modifications (16-18). DNA from cell line B958, EBV transformed human lymphocytes (American Type Culture Collection), was used as positive control and double distilled water as negative control.

Duplex PCR was performed to detect EBV by two sets of primers. The first amplified the non-polymorphic EBV nuclear antigen 1 (EBNA-1), generating an approximately 610 bp DNA fragment. The second amplified a human β-actin genomic sequence, generating an approximately 318 bp DNA fragment. The oligonucleotide sequences for both sets of PCR primers were identical to the ones previously reported (16).

Two sets of PCR primers were used for EBV typing. The first primer amplified the EBV nuclear antigen 2 (EBNA-2), generating a DNA fragment of 168 bp for EBV type A and of 184 bp for EBV type B. The second one amplified the EBV nuclear antigen 3C (EBNA-3C), generating a DNA fragment of 153 bp for EBV type A and of 246 bp for EBV type B. The oligonucleotide sequences for both sets of PCR primers were identical to the ones previously reported (17,18).

The PCR reactions were performed in a total volume of 20 μl using 50 ng of the respective tumor DNA in 200 μM dNTP each, 1.5 mM MgCl2, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 0.5 units of Thermus aquaticus DNA polymerase (Promega) and 0.5 μM of each primer. The PCR amplifications were performed as follows: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 57 °C for 30 sec, with an extension at 72 °C for 1 min and a final extension at 72 °C for 7 min. The PCR products were then analyzed by 2% agarose gel electrophoresis.

Allelotyping

Fifty six microsatellite markers for PCR analysis are listed in table 1. For each chromosomal arm one to five markers were tested.

One strand of each primer pair was end-labeled at 37 °C for 1-2 hours in a total volume of 10 μl containing 10 μM primer, 0.025 mCi [32P] γATP (Amersham) at 3,000 Ci/mmol, 10 mM MgCl2, 5 mM DTT, 70 mM Tris-HCl (pH 7.6), and 10 units of T4 polynucleotide kinase (New England Laboratories). Without further separating the unincorporated nucleotides, the kinase reaction was added to the PCR buffer mix.

The PCR reactions were performed in a total volume of 10 μl using 50 ng of genomic DNA in 200 μM dNTP each, 10 mM TrisHCl (pH 8.4), 50 mM KCl, 1.5 mM MgCl2 (for all reactions; except NF1 and D20S470, 2.5 and 2.0 mM MgCl2, respectively was added), 0.5 units of Thermus Aquaticus DNA polymerase (Perkin Elmer Cetus) and concentration calculated from 0.05-0.5 μM of each primer.

The marker sets of (D3S1600, D3S966, D9S169), (D11S534, GABRB3, D9S51, D10S169), (GLUT2, D2S102, TCRD), (IL2RB, D8S88), (D16S287, D4S174), (D21S258, MFD133, D20S17, D1S103), (D12S341, D19S221), and (D2S131, D10S249) were analysed for LOH by multiplex PCR. The others were amplified as simplex PCR (19).

Several PCR reactions, indicated in parentheses in table 1, have been optimized for each primer set as follows: for reaction 1 and 3; the initial denaturation step at 95 °C for 4 min, then followed by 25 cycles of denaturation at 94 °C for 1 min, with 1 min annealing at 55 °C for reaction 1, or 52 °C for reaction 3, extension at 72 °C for 2 min and a final extension at 72 °C for 7 min.

For reaction 2, the initial denaturation step was 95 °C for 4 min, then followed by 5 cycles of step-down PCR denaturation at 94 °C for 1 min, with 1 min annealing at 60 °C, 59 °C, 58 °C, 57 °C, and 56 °C respectively, extension at 72 °C for 2 min and 25 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, with extension at 72 °C for 2 min and a final extension at 72 °C for 7 min.

Two μl of each reaction were mixed with 1 μl formamide-loading buffer, heated at 95 °C for 2 min, put on ice for 30 sec and then loaded onto 6% polyacrylamide/7 M urea gel. DNA fragments were size-fractionated at 70 Watts until the tracking dye reached the appropriate point on the gel. After electrophoresis, the wet gel was tranferred to filter paper (Watman), wrapped with Saran wrap and exposed to Kodak T-mat X-ray film for 6-24 hrs at -70 °C with an intensifying screen.

Results

Twenty seven EBV-associated-NPC samples were selected for LOH studies. All biopsied specimens were histologically confirmed. Among these 27 cases, 15 were WHO type II and the others were WHO type III. Twenty six cases were infected with EBV subtype A and one case with type B (Figure 1 and Table 1).

LOH in NPC

A panel of 56 microsatellite polymorphic markers representing every chromosomal arm was used to screen for LOH frequency. Table 1 shows the polymorphic loci used to test each chromosomal arm, patient staging, EBV typing and LOH, as well as microsatellite instability (MSI) results. Results representative for LOH are shown in figure 2. Frequencies of LOH for each autosomal arm are represented in figure 3.

The frequencies of LOH from each chromosomal arm varied from 0 to 87%. Chromosome 3p and 9p with 78 and 87%, respectively revealed higher incidence than other chromosomal arms. For chromosome 3p, further analysis displayed that there were at least two LOH loci which were 3p14, D3S1600 and 3p25, D3S192 and D3S1038. Two cases, 51 and 70, showed LOH from 3p25 but not 3p14. In contrast, two cases, 47 and 50, revealed loss from 3p14 but not 3p25. Two cases, 35 and 93, presented loss from both 3p14 and 3p25 but not 3p21. Thus, 3p14 and 3p25 were two separate LOH loci. Other regions with allelic loss over 50% were on chromosome 9q (50.0%), 11q (53.8%), 13q (63.6%) and 14q (52.6%). Further analyses of chromosome 11q revealed higher frequency of LOH on 11q23 (53.8%), while only 7 out of 27 (25.9%) had LOH on 11q13. Additional analyses on chromosome 13 displayed that the incidence of LOH for D13S284, located on 13q14, was higher than D13S119, located on 13q14.3-q22. LOH between 35 and 50% were noted on chromosome 3q (47.8%), 16q (47.8%), 19p (36.0%), and 22q (45.5%). Finally, several other chromosomes demonstrated allelic loss in 20 to 35%.

MSI in NPC

Out of 27 samples tested, MSI for multiple loci was presented in only one case, i.e., 38. MSI was revealed in 25 from 56 loci. In addition, sample 93 demonstrated MSI on two loci, D11S956 and D18S35, and sample 53 showed MSI on one locus, D11S956. Representative results for MSI are shown in figure 4. No significant clinical difference was noted regarding these cases.

Discussion

Several areas of chromosomal loss during cancer development and progression are associated with inactivation of both tumor suppressor gene alleles (5, 7, 13). In addition, they are correlated with the histopathology, staging and clinical outcome of cancer (20). Here we demonstrated several chromosomes with significant LOH in NPC. While several LOH loci are common to HNSCC, some appear to be unique to NPC progression.

Previously Choi *et al.*, showed LOH on chromosome 3p for all of informative 35 NPC cases and Huang *et al.*, studied chromosome 9p and found allelic loss on 11 samples from 18 NPC (6,8). Consistently, in this study two of the highest incidences of allelic loss have been shown on chromosomes 3p (78%) and 9p (87%). Three LOH loci have been reported on chromosomes 3p, 3p14, 3p21 and 3p25 (21). This study revealed that at least the 3p14 and 3p25 loci are associated with NPC development. It should also be noted that a putative tumor suppressor gene, VHL, is the candidate gene on chromosome 3p25 (22). For 9 p, the LOH locus has been defined on chromosome band 9p21 and gene p16, controlling cell cycles, has previously been shown to have homozygous deletion or hypermethylation (10,11). Interestingly, 9pLOH has now been well documented in precancerous lesions of HNSCC (12). It is interesting to investigate whether genetic alterations of chromosome 9p might also be an early event of NPC carcinogenesis.

Allelic loss of chromosome 11q has been observed in several other tumor types, such as HNSCC, breast, ovary and lung (13, 23, 24). Poor prognosis of breast and ovarian cancer has been associated with 11qLOH (23). At least three LOH loci have been delineated on chromosome 11q, 11q13, 11q15 and 11q23 (24). In addition, 11q13 is a chromosome region with high frequency of amplification in HNSCC (25). This results in allelic imbalance and may be difficult to distinguish from LOH. This study described a higher frequency of allelic loss in NPC on 11q23 than 11q13. It should also be noted that the LOH on 11q23 may be related to the ataxia-telangiectasia locus (26).

Chromosome 13qLOH is also frequently detected in several cancers, such as retinoblastoma, breast cancer, and HNSCC (5,27,13). This study has shown that the common LOH locus in NPC may be located proximal to 13q14.3. At least two tumor suppressor genes are located proximal to this region. The first is Rb, retinoblastoma gene, and the other is BRCA2, the candidate gene for the second locus of the familial breast cancer syndrome (5,27). Previous mutation analysis of the Rb on NPC demonstrated negative results (28). Thus it is tempting to hypothesize that the BRCA2 tumor suppressor gene may be responsible for NPC development.

The allelic loss of chromosome 14q was also frequently found in other types of cancer, for example bladder cancer, neuroblastoma, colorectal cancer and HNSCC (13,29-31). A recent study has delineated two tumor suppressor gene loci on chromosome 14 i.e., 14 q12 and 14q32 (29). Interestingly, LOH of 14q is associated with an advanced phenotype of neuroblastoma and frequently found in advanced colorectal cancer (30,31).

Chromosome 17p is one of the most common regions with genetic alterations reported in cancer. P53, the best known tumor suppressor gene, is located on this chromosome (32). P53 alterations including protein expression and mutations are common in HNSCC while mutation of p53 in NPC is infrequent (33-36). The LOH study of this chromosome supported the mutation data in which allelic loss of chromosome 17p was noticed in only 30% of the NPC while previous reports revealed 50% LOH of the HNSCC (13,14).

Among 27 cases tested, 15 were WHO type II and 12 were WHO type III. The average LOH per each chromosome regions were 0.34 and 0.31 for WHO type II and III respectively. Interestingly, LOH were found more frequent on chromosome 4p, 7p, 9q, 11q and 22q for WHO type II while higher frequency of LOH for WHO type III were reported on

chromosome 6p and 15q. However, because of the limited number of tumors, these comparative datas have not yet been statistically significant.

MSI is presented as variations in the length of microsatellite repeats in tumor DNA when compared with matched normal DNA. The abnormality in size of microsatellite loci has been observed in various types of cancer as well as in hereditary non-polyposis colorectal cancer (HNPCC) (37). In HNPCC, mutations in a number of DNA mismatch repair genes *(hMSH2, hMLH1, hPMS1*, and *hPMS2*), have been reported. Thus, MSI may be the consequence of decreased accuracy of the DNA mismatch repair system during DNA replication which might facilitate the accumulation of mutations (38). This study has presented MSI of multiple loci in only one out of 27 NPC samples tested. This suggests that the phenomenon of MSI is a relatively rare event during NPC development.

NPC is a unique subclassification of HNSCC due to its endemic distribution and etiologic co-factors. It would be of great interest and importance to elucidate whether the genetic events in Epstein-Barr-Virus-associated NPC are similar or distinct from HNSCC. Previous HNSCC studies demonstrated high percentage of LOH on chromosomes 3p, 3q, 6p, 8p, 8q, 9p, 11q, 13q, 14q, 17p, 18q and 19q (13 Nawroz *et al.*, 1994; 14 Field *et al.*, 1995; 12 El-Naggar *et al.*, 1995). Nawroz *et al.*, studied 29 HNSCCs and showed 67, 50, 38, 40, 38, 72, 61, 54, 39, 52, 23 and 40 percent of allelic losses respectively. Additionally, Field *et al.*, tested 80 specimens and found LOH more frequently on chromosome 3p, 8p, 9p, 13q, 17p, 18q and 19q for 52, 35, 62, 27, 50, 49 and 29 percent respectively. Finally, El-Naggar *et al.*, studied 20 patients for LOH on chromosome 3p, 5q, 8p, 9p, 9q, 11q and 17p and high incidence of LOH in invasive carcinoma was observed at 9p (72%), 8p (53%), 3p (47%), 9q (35%), and 11q (33%). Similar incidences on chromosomes 3p (78%), 3q (48%), 6p (48%), 9 p (87%), 11q (54%), 13q (64%), and 14q (43%) have also been detected regarding NPC. However, NPC revealed lower incidences of LOH on chromosomes 8p (13%), 17p (30%) and 19q (11%). On the contrary, this study has shown frequent allelic loss regarding NPC on chromosomes 16q (48%) and 22q (46%). Since several genetic alterations of NPC and HNSCC are similar, the multistep processes for development and progression of both cancers overlap. However, some genetic changes seems to be unique in biology of NPC development.

It would be also interesting to compare these allelotyping datas with allelic loss pattern on other EBV associated neoplasias for example post immunosuppression/transplant, AIDS related, and Burkitt's lymphomas. However, there is only limited knowledge of LOH for comparison at present.

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Table 1.a *LOH and MSI data for each locus of 27 NPC*

	Locus (C)[a]	Location	L/I(%)	11	18	19	24	31	35	38	44	45	47	50	51	53	56	67	70	76	77	83	86	93	102	103	105	112	123	18
Stage				III	IV	IV	IV	R	IV	II	III	IV	IV	IV	IV	III	IV	IV	IV	IV	IV	IV	III	I	IV	IV	IV	IV	IV	III
T				3	3	2	4		4	2	2	1	4	4	3	3	3	2	4	2	2	3	3	4	4	3	2	3	4	3
N				0	2b	2c	3		3	0	1	2b	2b	0	3	1	3	2a	2c	2b	3	3	0	1	2c	2c	2c	2b	0	0
M				0	0	0	0		1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
WHO				II	II	II	II	II	II	II	II	II	II	II	II	II	II	II	II	II	II	II	II	II	III	III	III	II	III	II
EBV				A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A	A	A
	D1S243 (1)	1p36.1-2	7/24 (29.2)	-	-	u	-	+	-	+	-	u	-	-	+	-	+	-	-	-	-	-	-	u	+	+	+	-	-	-
	D1S103 (1)	1q31q32	5/25 (20.0)	+	+	-	-	-	-	i	-	-	-	u	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
	D2S131 (3)	2p	2/20 (10.0)	-	u	-	-	-	-	i	-	-	-	u	-	-	+	u	-	-	-	-	u	-	+	u	u	-	-	-
	D2S102 (1)	2q33q37	5/22 (22.7)	-	+	-	-	+	-	i	+	-	u	-	+	u	-	-	-	-	-	u	-	+	-	-	u	-	-	-
	D3S1038 (3)	3p25	18/24 (75.0)	u	-	u	+	+	+	+	+	u	-	-	u	+	+	u	+	+	+	+	+	+	u	+	+	u	u	u
	D3S192 (3)	3p25		u	-	u	+	n	n	+	+	-	-	-	+	+	+	u	+	u	u	+	+	+	+	u	u	+	-	-
	D3S966 (1)	3p21.3	12/18 (66.6)	-	u	+	+	+	-	i	+	u	u	+	+	+	+	-	-	-	u	+	u	-	+	+	u	+	u	n
	D3S1600 (1)	3p14	15/20 (75.0)	u	-	u	+	+	+	i	+	-	+	+	-	+	+	+	-	-	+	+	+	+	+	+	u	u	u	n
	GLUT2 (1)	3q26.1-2	11/23 (47.8)	u	+	+	-	-	-	i	+	+	u	u	u	u	u	u	+	-	u	-	+	+	+	-	-	+	-	u
	D3S1744 (1)	3q23q24		-	+	u	n	n	-	i	+	+	n	-	+	n	-	n	n	n	+	-	u	+	+	-	-	+	-	-
	D4S174 (1)	4p11p15	6/23 (26.1)	-	-	-	-	-	-	i	+	u	-	+	+	+	+	-	-	u	-	+	-	-	u	-	-	-	-	-
	D4S1554 (2)	4q11q35	4/23 (17.4)	-	u	+	n	-	+	i	-	-	-	-	+	u	-	-	-	-	-	-	-	-	-	+	-	-	-	-
	D5S392 (1)	5p	6/25 (24.0)	+	-	n	n	-	n	+	-	u	n	u	-	n	-	-	n	+	-	+	-	u	-	+	-	-	-	-
	D5S819 (2)	5p		u	u	-	n	u	-	-	u	u	-	-	-	-	-	u	-	u	u	n	u	-	+	+	-	-	-	n
	D5S82 (1)	5q14q21	7/21 (33.3)	-	-	+	+	-	u	i	+	u	u	u	+	-	-	+	-	-	-	-	-	-	+	+	-	u	-	-
	D6S309 (2)	6p	10/21 (47.6)	+	-	+	+	-	u	i	+	-	+	-	+	-	-	u	+	-	+	u	u	+	+	-	u	-	-	-
	D6S503 (3)	6q	7/22 (31.8)	-	-	-	u	u	+	-	-	-	u	-	-	+	u	-	-	-	+	+	-	u	-	+	-	+	+	-
	D7S517 (1)	7p	4/24 (16.7)	-	+	-	-	-	-	+	+	-	-	-	-	+	-	-	u	-	-	-	u	-	-	u	-	-	-	-
	D7S486 (2)	7q31	6/26 (23.1)	-	-	-	-	-	-	+	+	-	-	-	-	+	u	-	+	-	-	+	-	+	-	-	-	-	-	-

Table 1.b *LOH and MSI data for each locus of 27 NPC*

Locus (C)[a]	Location	L/I (%)	11	18	19	24	31	35	38	44	45	47	50	51	53	56	67	70	76	77	83	86	93	102	103	105	112	123	18
NEFL (1)	8p	3/23 (13.0)	-	-	-	u	-	u	+	-	-	-	-	-	+	-	u	-	-	-	-	u	-	+	-	-	-	-	-
D8S88 (1)	8q22	3/22 (13.6)	-	-	-	u	-	-	i	-	-	u	-	-	-	-	+	-	-	-	u	+	-	-	-	+	-	u	-
D9S169 (1)	9p21	20/23 (87.0)	+	-	+	+	+	-	+	+	+	+	+	-	+	u	+	u	u	u	n	u	+	+	+	u	u	u	+
IFNA (2)	9p22		n	-	u	n	n	-	u	+	+	+	+	-	+	+	+	u	+	+	+	u	u	+	+	u	+	u	+
D9S51 (1)	9q	11/22 (50.0)	-	+	+	-	+	u	+	-	-	+	u	-	-	+	+	-	u	u	-	-	u	u	u	u	u	u	1
ABL1 (1)	9q34		n	+	+	u	u	u	u	u	u	n	u	-	+	u	n	u	u	u	u	-	u	-	u	u	u	+	-
D10S249 (3)	10p	4/21 (21.1)	-	-	-	n	-	-	-	-	-	+	-	+	+	-	-	-	u	-	n	-	u	u	+	u	-	-	-
D10S169 (1)	10q11.2	3/22 (13.6)	u	-	-	n	u	u	i	-	u	+	u	u	u	u	-	u	u	-	+	+	-	u	u	u	u	u	:
D10S677 (1)	10q		-	n	n	n	-	-	-	-	-	n	-	-	n	-	n	-	n	-	+	+	-	u	-	-	n	-	
WT1 (1)	11p13	7/25 (28.0)	u	u	+	n	-	-	+	u	+	-	u	+	+	-	-	-	u	u	u	-	u	u	-	u	-	u	i
D11S554 (2)	11p		-	-	-	n	n	-	u	+	-	-	u	-	n	-	n	-	-	-	-	-	-	+	-	-	-	-	-
D11S534 (1)	11q13	7/27 (25.9)	u	-	-	n	-	u	i	-	-	u	u	-	u	+	u	u	-	-	-	u	-	-	-	u	u	u	-
D11S956 (1)	11q13		u	-	u	u	n	-	+	-	-	-	-	i	+	+	+	-	-	n	n	-	i	-	u	+	u	-	-
INT2 (1)	11q13.3		-	-	-	-	-	u	i	-	u	-	u	-	u	+	+	-	-	-	-	-	-	-	-	u	+	-	-
D11S976 (1)	11q23	14/26 (53.8)	u	-	u	n	n	+	+	+	+	-	+	u	u	+	n	+	n	+	n	-	-	+	u	+	+	-	+
D11S897 (2)	11q23		-	-	-	-	-	u	+	+	+	-	u	-	+	u	+	+	-	+	u	-	u	+	-	+	+	-	-
D12S341 (2)	12p	7/23 (30.4)	-	-	-	+	+	-	i	u	-	-	+	-	+	-	-	+	-	-	n	-	-	n	+	+	-	-	-
MFD133 (1)	12q	4/20 (20.0)	u	-	-	+	-	-	i	+	-	-	+	-	-	u	-	-	-	u	-	-	u	+	u	-	-	-	1
D13S284 (2)	13q14.2	14/22 (63.6)	n	+	+	+	+	-	i	+	-	+	-	+	+	+	+	-	-	u	-	-	u	+	+	+	-	+	1
D13S119 (1)	13q14.3-q22	6/20 (30.0)	-	u	+	n	-	u	-	+	u	u	u	-	-	-	-	u	-	-	-	-	-	+	+	+	-	-	-
TCRD (1)	14q11.2	10/19 (52.6)	-	u	u	u	+	u	u	u	+	-	+	-	+	+	+	+	-	-	+	+	u	+	u	-	-	-	-
D14S118 (1)	14q	6/14 (42.9)	u	+	u	n	-	+	u	+	u	-	u	-	+	+	u	-	+	-	u	-	u	u	u	u	u	u	-
GABRB3 (1)	15q11q13	4/22 (18.2)	-	-	u	n	-	+	i	-	-	u	-	-	-	-	-	-	-	-	-	u	+	+	+	-	-	-	-
D16S287 (1)	16p13.11	4/22 (18.2)	-	u	-	+	+	-	-	-	-	-	+	+	n	-	u	-	u	-	-	-	-	-	u	-	-	-	-
D16S511 (2)	16q22q24	11/23 (47.8)	-	-	-	-	+	+	i	+	+	u	-	+	+	-	-	-	-	-	-	+	+	+	+	-	-	u	+

Table 1.c *LOH and MSI data for each locus of 27 NPC*

Locus (C)[a]	Location	L/I (%)	11	18	19	24	31	35	38	44	45	47	50	51	53	56	67	70	76	77	83	86	93	102	103	105	112	123	8
D17S520 (1)	17p12	8/27 (29.6)	-	-	-	-	-	-	i	-	-	-	-	-	+	+	u	-	-	+	u	u	-	+	+	+	-	-	
D17S1176 (2)	17p		-	-	-	-	-	-	+	-	-	-	-	-	+	+	-	-	-	+	-	+	-	+	+	u	u	-	
KRT9 (2)	17q21	6/19 (31.6)	+	u	-	-	-	-	i	-	-	+	-	+	+	+	-	-	-	+	n	u	-	u	u	u	-	u	
D18S59 (1)	18p11.2	0/20 (0)	u	-	-	-	-	-	-	-	-	-	-	-	u	-	-	-	-	u	-		u	-	-	-	u	-	
D18S35 (1)	18q21.2	6/24 (25.0)	u	u	-	+	-	u	+	-	+	-	u	-	n	+	+	-	u	-	u	-	i	-	u	u	u	-	
DCC (1)	18q21.1		u	-	u	+	-	+	i	u	-	-	-	u	-	+	u	-	-	-	n	u	-	u	-	-	u	-	
D19S221 (2)	19p	9/25 (36)	-	+	-	u	-	-	-	+	+	-	-	-	+	-	+	-	+	-	-	+	+	+	u	-	-	u	
D19S412 (2)	19q	2/19 (10.5)	u	-	-	u	u	-	i	-	u	-	u	+	-	-	-	+	-	-	-	-	-	-	-	u	-	-	
D20S470 (3)	20p	4/20 (20.0)	-	+	-	n	-	u	i	-	+	u	u	-	+	-	-	-	u	-	-	-	-	-	+	u	-	-	
D20S17 (1)	20q12	3/20 (15.0)	-	-	u	+	-	-	u	+	-	-	u	-	+	-	u	-	-	u	-	-	u	-	-	-	-	u	
D21S258 (1)	21q	3/20 (15.0)	-	u	u	u	-	-	-	+	u	-	-	-	+	-	u	+	-	-	u	-	-	-	-	u	-	-	
IL2RB (1)	22q	10/22 (45.5)	u	-	+	n	+	-	+	-	+	-	-	+	+	+	+	+	-	-	u	u	+	-	-	-	-	u	

[a] C, PCR condition; L, number of positive LOH cases; I, number of informative cases; R, recurrence; A, EBV type A; B, EBV type B; +, positive LOH result: -, negative LOH result; I, microsatellite instability; u, uninformative result; n, not done.

Figure legends

Figure 1. Autoradiographs showing PCR genotyping of EBV infected NPC on a 2% agarose gel stained with ethidium bromide. The first lane from the left is ϕ174 HaeIII standard DNA size marker. + and - are PCR products from positive controls, B958 cell line, and negative controls, double distilled water, respectively. Numbers indicate corresponding PCR products from NPC patients. (A) Duplex PCR generating 610 bp and 318 bp DNA fragments for EBNA-1 and human b-actin genomic sequence, respectively. (B) PCR generating 246 bp or 153 bp DNA fragments for EBNA-3C of EBV type B or type A, respectively. (C) PCR generating 184 bp or 168 bp DNA fragments for EBNA-2 of EBV type B or type A, respectively.

Figure 2. Autoradiographs showing LOH-analysis using microsatellite markers. Representative NPC tumours (T) and corresponding normal leukocytes (N) are shown with microsatellite markers indicated on the bottom. Markers D9S169, D11S897, and D13S284 reveal loss of upper alleles and markers D3S1038 and TCRD reveal loss of lower alleles.

Figure 3. Frequency of allelic loss for autosome in NPC. Allelotyping was accomplished using polymorphic microsatellite analysis. The probes used are listed in Table 1.

Figure 4. Autoradiograph showing the microsatellite instability at KRT9 and DCC loci. N, normal DNA ; T, tumor DNA.

Figure 1

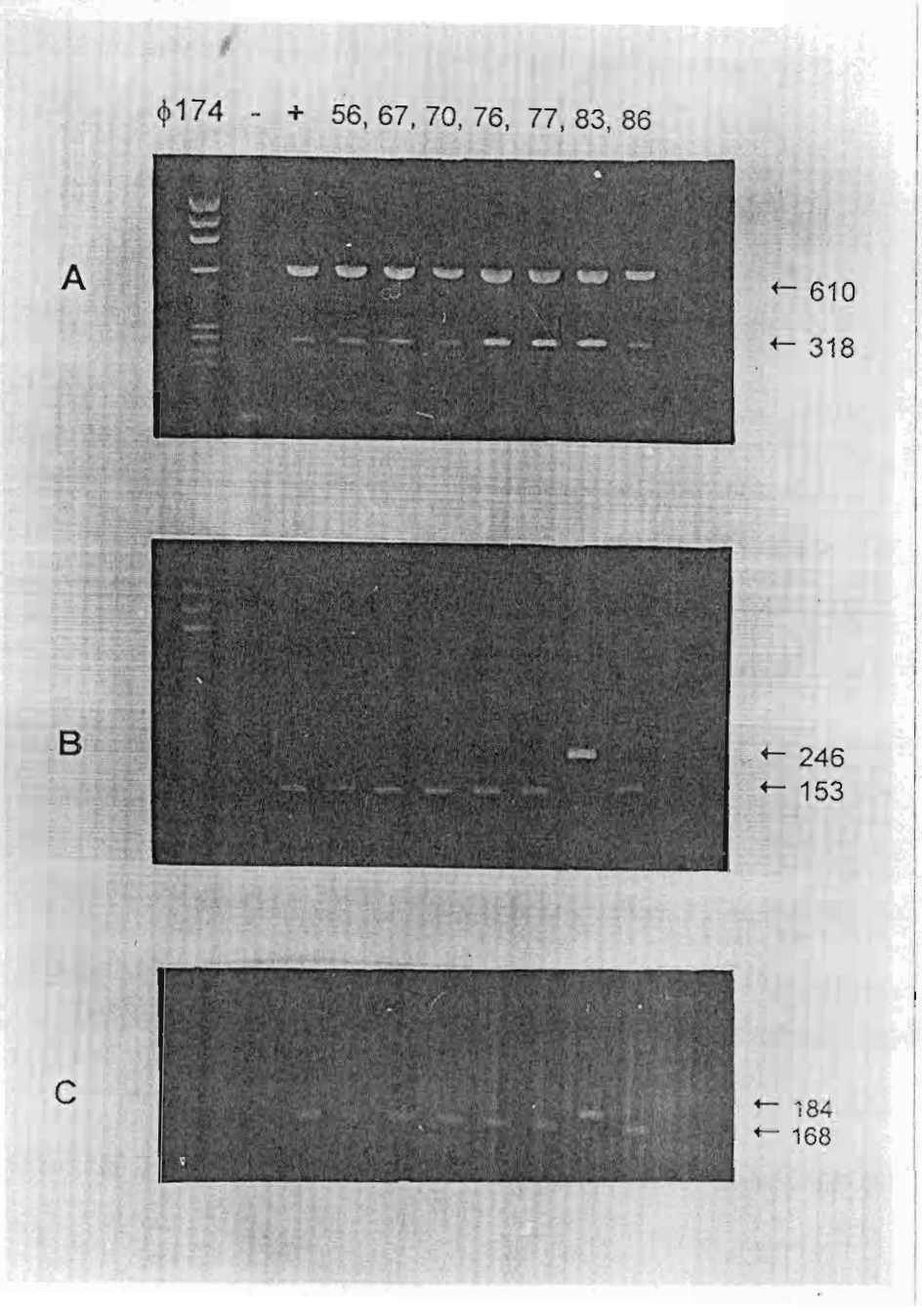


Figure 2

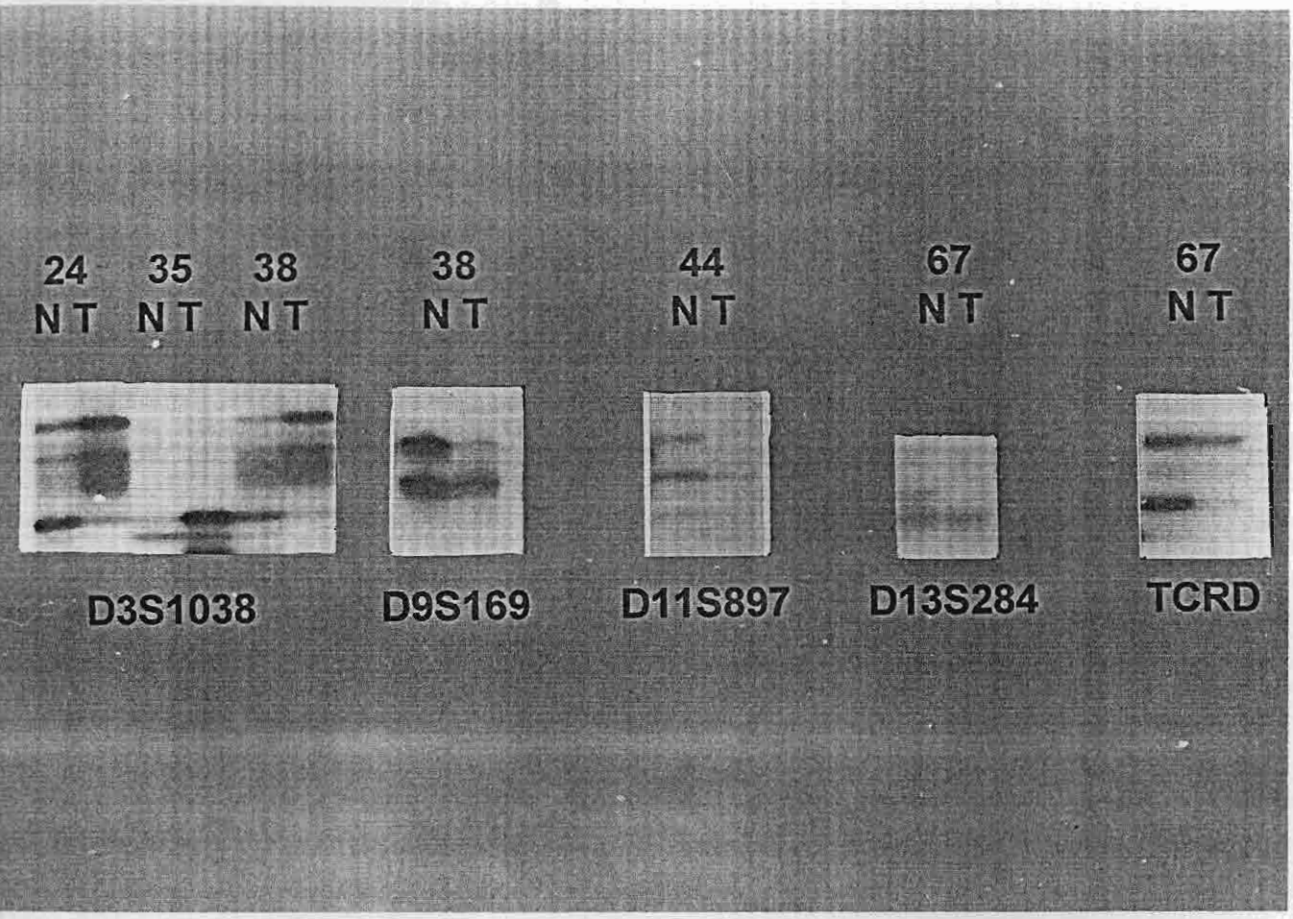

24
NT
35
NT
38
NT
38
NT
44
NT
67
NT
67
NT
D3S1038
D9S169
D11S897
D13S284
TCRD

Figure 3

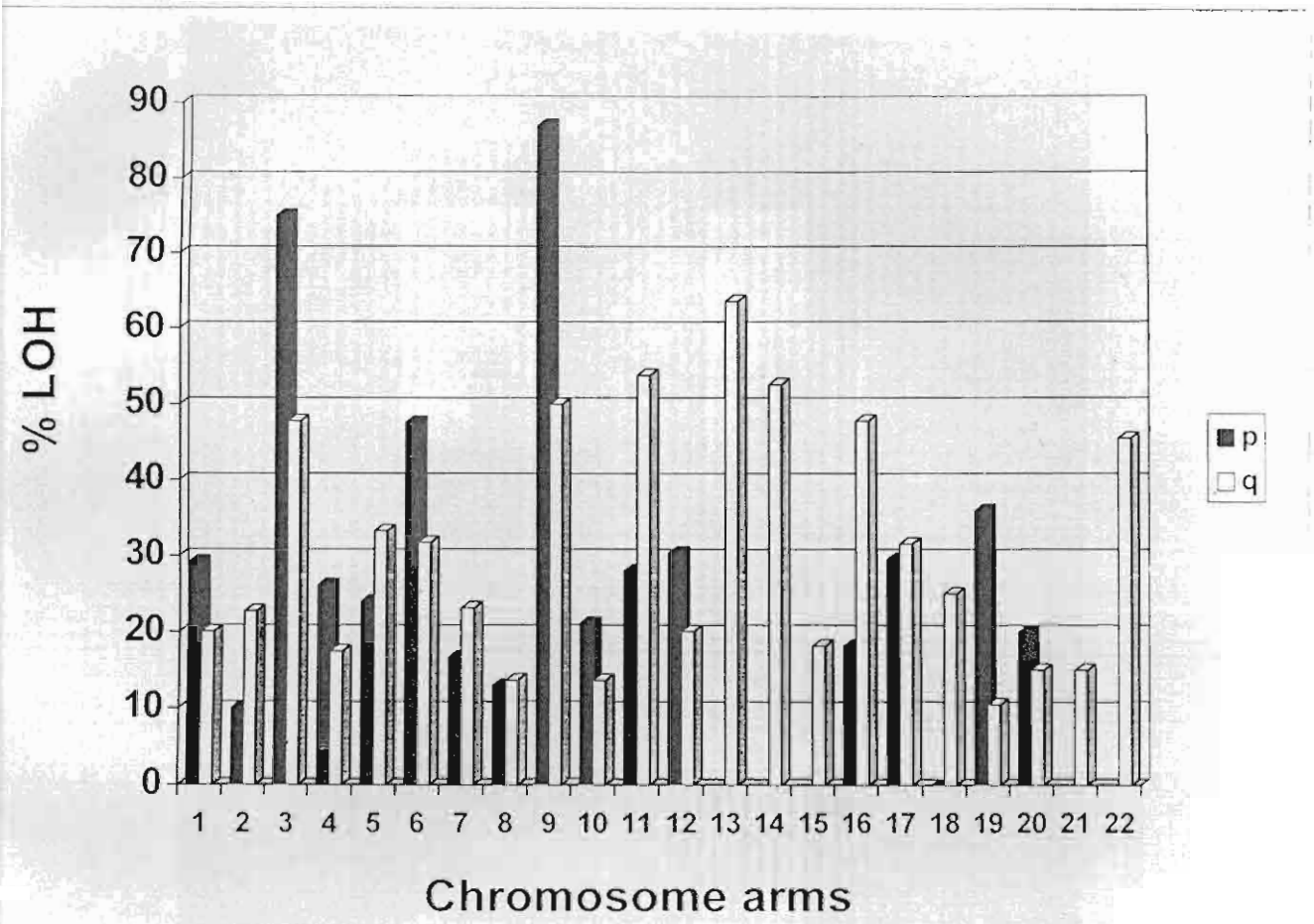
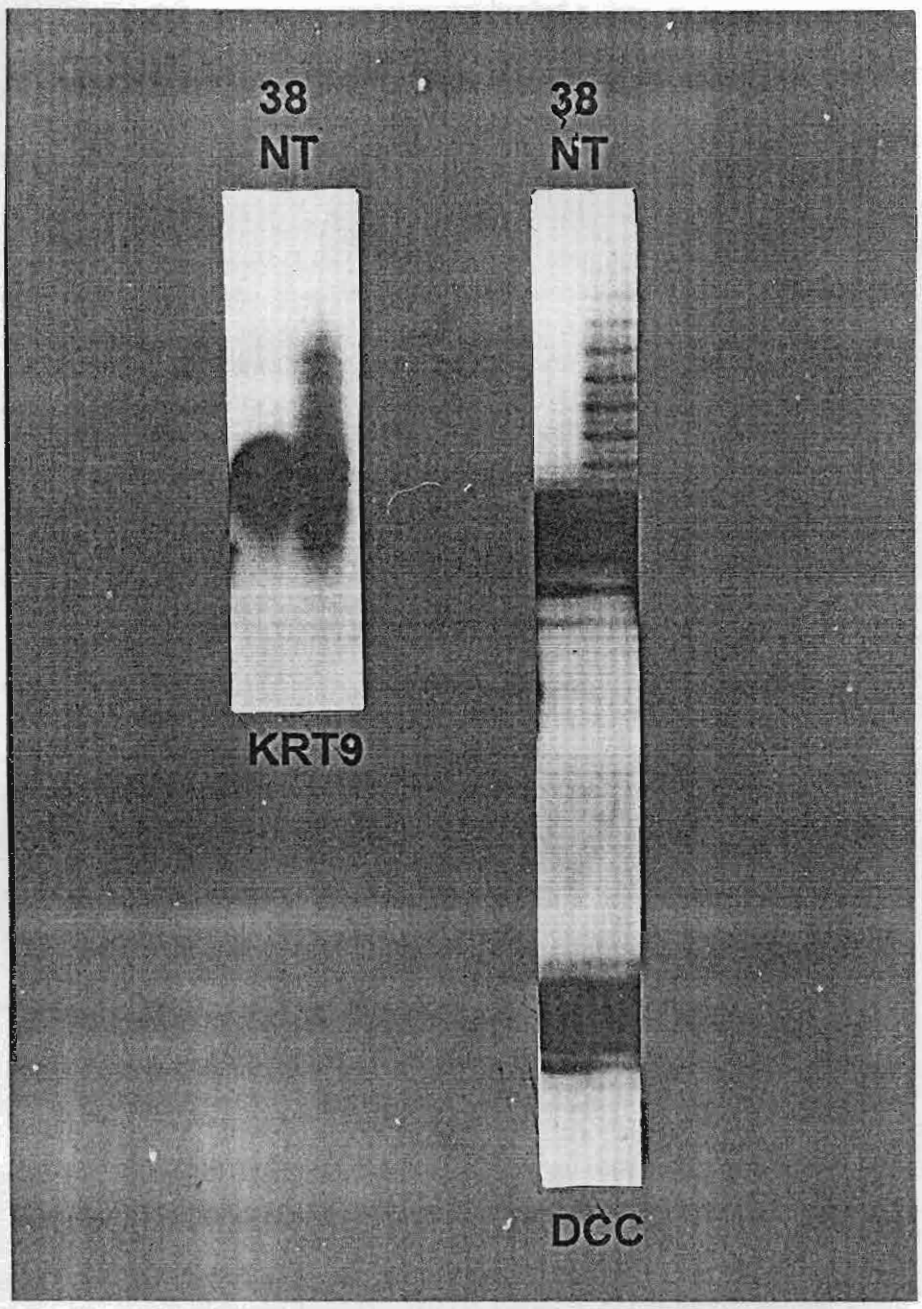

% LOH
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Chromosome arms
p
q

Figure 4



Chapter 2

Loss of Heterozygosity on Chromosome 14 in Nasopharyngeal Carcinoma

This results presented in this chapter have been taken, in part, from a published manuscript

- **Mutirangura A**, Pornthanakasem W, Sriuranpong V, Supiyaphun P and Voravud N. Loss of heterozygosity on chromosome 14 in nasopharyngeal carcinoma. Cancer Research (submitted).

Loss of heterozygosity (LOH) is a result of common genetic changes in epithelial solid tumors and reflects the loss of tumor suppressor gene function in that region (6). Consequently, careful mapping of these somatic losses is more likely to reveal the location of critical tumor suppressor genes. We previously reported the incidence of LOH in NPC to amount to a frequency of loss on chromosomes 3, 9, 11q, 13q and 14q exceeding 50% (5). Detailed mapping of chromosomes 3, 9 and 11q, but not 14q LOH, has been previously reported (7-9). In the present study, we identified the frequency and minimal region of 14q loss in NPC.

Chromosome 14qLOH has been shown to be related to the development of several forms of cancer, including meningioma, renal cell carcinoma, neuroblastoma and stomach cancer (10-13). Two previous reports on primary bladder cancer and ovarian carcinoma defined LOH mapping on chromosome 14 at 14q12-13 and 14q32 (14,15). In addition, translocations involving chromosome 14 were commonly found in hematologic malignancies (16-18). These translocations frequently involved 14q11 and were probably mediated by illegitimate V(D)J recombination.

In this study, we defined three minimal regions of loss on chromosome 14. Two regions confirmed that there were at least two tumor suppressor genes on this chromosome. The third region of LOH was specific to the T-cell receptor delta chain locus (TCRD). This may suggest that there is another tumor suppressor gene locus linked to TCRD or the LOH may be a result of frequent chromosomal rearrangement of this region.

Materials and Methods

Tissues and DNA Extraction.

Primary NPC tissues were collected from 40 patients prior to treatment at the Chulalongkorn University Hospital. The tissues from each patient were divided into two pieces. The first part was sent for routine histological examination. The second part was immediately stored in liquid nitrogen until further use. All the tumors were histologically ascertained to be undifferentiated NPC according to the WHO classification. The 40 tumors included stages ranging from II to IV. Blood samples obtained by venipuncture from the same patients were used as constitutional controls. DNA was extracted from the tumor tissues and blood leukocytes by methods previously described (19).

PCR amplification

LOH on chromosome 14q was analysed using the following 16 microsatellite markers: TCRD, D14S283, D14S80, D14S278, D14S75, D14S288, D14S285, D14S52, D14S274, D14S258, D14S67, D14S68, D14S256, D14S81, D14S51, and D14S267 (14,20). Information regarding cytogenetic localization of the markers was obtained from the Genome

Data Base at http://gdbwww.gdb.org. All primers for PCR amplification of dinucleotide microsatellite markers were obtained from Research Genetics (Huntsville, AL).

One strand of each primer pair was end-labeled at 37 °C for 1-2 hours in a total volume of 10 μl containing 10 μM primer, 0.025 mCi [32P] γATP (Amersham) at 3,000 Ci/mmol, 10 mM $MgCl_2$, 5 mM DTT, 70 mM Tris-HCl (pH 7.6), and 10 units of T4 polynucleotide kinase (New England Laboratories). Without further separating the unincorporated nucleotides, the kinase reaction was added to the PCR buffer mix.

The PCR reactions were performed in a total volume of 10 μl using 50 ng of genomic DNA in 200 μM dNTP each, 10 mM TrisHCl (pH 8.4), 50 mM KCl, 1.5 mM MgCl2, 0.5 units of Thermus Aquaticus DNA polymerase (Perkin Elmer Cetus) and primer concentrations between 0.05 and 0.5 μM each.

Several PCR reactions have been optimized as follows: An initial denaturation step at 95 °C for 4 min, followed by 25 cycles of denaturation at 94 °C for 1 min, with 1 min annealing at 55 °C, extension at 72 °C for 2 min and a final extension at 72 °C for 7 min.

Two μl of each reaction were mixed with 1 μl formamide-loading buffer, heated at 95 °C for 2 min, put on ice for 30 sec and then loaded onto a 6% polyacrylamide/7 M urea gel. DNA fragments were size-fractionated at 70 Watts until the tracking dye had covered the appropriate distance on the gel. After electrophoresis, the wet gel was tranferred to filter paper (Whatman), wrapped with Saran wrap and exposed to Kodak T-mat X-ray film for 6-24 hrs at -70 °C with an intensifying screen.

For each marker, LOH was scored by comparing the ratio of the signal intensities obtained per allele between tumor and normal DNA in heterozygous individuals. The criteria and rationale for the quantitative assessment have been described elsewhere (14).

Results

Allelic losses at one or more loci on chromosome 14q were observed in 29 of 40 (72.5%) nasopharyngeal carcinomas. There was no correlation between either the extent or chromosomal region of loss and histological subtype, stage, lymph node involvement, local invasion and distant metastasis. Of the 29 tumors with allelic loss, monosomy was observed in 5 cases and evidence of microsatellite instability was observed in two cases. The remaining 22 cases with partial loss were used to define minimal regions of deletion (Fig 1). These data are summarized in Fig 2.

Three minimal regions of loss were identified by this analysis (Fig 2). Four cases, 50, 78, 113 and 183, showed a specific deleted region within the TCRD locus. The second specific region of LOH was between D14S278 and D14S288 as shown in cases 45, 51 and 162. The third specific region of LOH was located in a more distal region as shown in 9 cases, 19, 44, 50, 57, 83, 93, 105, 162, and 188. The most minimal region was between D14S51 and the telomere as demonstrated in case 50. These three LOH regions have been cytogenetically mapped to chromosome 14q11, 14q12-13 and 14q32-qter, respectively (Fig 2).

Interestingly, while chromosome 14q breakage seems to occur randomly throughout, discordance of LOH between TCRD and D14S283 was detectable with high frequency (Fig 2, 3). Among 18 cases that were informative for both loci, seven (38.9%) showed discordance of LOH between TCRD and D14S283. Three of them, cases 78, 113 and 183, showed LOH for TCRD but not for D14S283 (Fig 2 and 3), but four cases, 51, 166, 189 and 197, demonstrated LOH for D14S283 but not TCRD (Fig 2). These data indicate that during NPC development chromosomal breakage between these two markers is a relatively common event.