Chromosome 3p12.3-p14.2 and 3q26.2-q26.32 Are Genomic Markers for Prognosis of Advanced Nasopharyngeal Carcinoma

Jim Jinn-Chyuan Sheu,1,4 Chia-Huei Lee,6 Jenq-Yuh Ko,7,8 George S.W. Tsao,11 Chung-Chun Wu,6 Chih-Yeu Fang,6 Fuu-Jen Tsai,2,5 Chun-Hung Hua,3 Chi-Long Chen,10 and Jen-Yang Chen6,9

Abstract

Purpose: Nasopharyngeal carcinoma is an epithelial malignancy with a remarkable racial and geographic distribution. Previous cytogenetic studies have shown nasopharyngeal carcinoma to be characterized by gross genomic aberrations. However, identification of susceptible gene loci in advanced nasopharyngeal carcinoma has been poorly discussed.

Experimental Design: A genome-wide survey of gene copy number changes was initiated with two nasopharyngeal carcinoma cell lines by array-based comparative genomic hybridization analysis. These alterations were confirmed by a parallel analysis with the data from the gene expression microarray and were validated by quantitative PCR. Clinical association of the defined target genes was analyzed by fluorescence in situ hybridization on 48 metastatic tumors.

Results: A high percentage of genes were consistently altered in dosage and expression levels with gain on 3q26.2-q26.32 and losses on 3p12.3-p14.2 and 9p21.3-p23. Six candidate genes, GPR160 (3q26.2-q27), SKIL (3q26), ADAMTS9 (3p14.2-p14.3), LRIG1 (3p14), MPDZ (9p22-p24), and ADFP (9p22.2) were validated by quantitative PCR. Fluorescence in situ hybridization studies revealed amplification of GPR160 (in 25% of cases) and SKIL (33%); and deletion of ADAMTS9 (30%), LRIG1 (35%), MPDZ (15%), and ADFP (15%). Clinical association analyses indicated a poor survival rate with genetic alterations at the defined 3p deletion (P = 0.0012) and the 3q amplification regions (P = 0.0114).

Conclusion: The combined microarray technologies suggested novel candidate oncogenes, amplification of GPR160 and SKIL at 3q26.2-q26.32, and deletion of tumor suppressor genes ADAMTS9 and LRIG1 at 3p12.3-p14.2. Altered expression of these genes may be responsible for malignant progression and could be used as potential markers for nasopharyngeal carcinoma.

(Cancer Epidemiol Biomarkers Prev 2009;18(10):2709–16)

Introduction

Nasopharyngeal carcinoma is a malignant tumor of epithelial origin. As one of the most common cancers in southern China and Southeast Asia (1), it causes a high incidence of mortality among the inhabitants of endemic regions. Genetic susceptibility (2-4), EBV infection (5), and chemical carcinogens (6, 7) are thought to be implicated in the incidence of fatality among the inhabitants of endemic regions. Genetic susceptibility (2-4), EBV infection (5), and chemical carcinogens (6, 7) are thought to be implicated in the onset of nasopharyngeal carcinoma. The remarkable racial distribution and the much higher risk of nasopharyngeal carcinoma in US-born Chinese, compared with Caucasians (8), suggest genetic predisposition may play a significant role in the onset of nasopharyngeal carcinoma. The aggravation of genomic aberrations signifies poor prognosis in nasopharyngeal carcinoma patients (9, 10), and the degree of genome instability is directly related to the invasiveness and tumorigenesis found for some nasopharyngeal carcinoma cell lines (11, 12). These results clearly indicate the profound influence of genetic aberrations on relapse and metastasis of this malignancy.

Nasopharyngeal carcinoma is characterized by gross numerical abnormalities of chromosomes, which frequently would result in the altered expressions of oncogenes and tumor suppressor genes. Conventional and molecular cytogenetic methods, such as comparative genomic hybridization (CGH) analysis and loss of heterozygosity studies have located multiple regions of chromosomal gains and losses in nasopharyngeal carcinoma. Gains of 1q, 2q, 3q, 4q, 6p, 7q, 8q, 11q, 12q, 12q, 15q, 17p, and 17q, and losses of 1p, 3p, 8p, 9p, 11q, 13q, 14q, and 16q have been found to occur with high frequency in tumor samples and cell lines (13-19). In addition, evolutionary tree models for this malignancy have been...
constructed based on CGH data. Loss of 3p and gain of 12p are important early events, and gain of 8q is a late event, in nasopharyngeal carcinoma progression (20, 21). Nevertheless, the limited resolution of the technologies precludes the identification of candidate genes. Thus, the molecular mechanism underlying carcinogenesis of nasopharyngeal carcinoma remains obscure.

Following the recent development of array-based CGH with higher mapping resolution, some studies successfully defined the regions of genomic imbalance to the cytoband level. Deletions in 3p13-p23, 11q14-q23, 13q12-q31, and 14q24-qter, and amplifications of 1p34.3, 1q25-qter, 3q26, 12p13, and 12q13 were detected in nasopharyngeal carcinoma genomes (22-24). However, the power to yield meaningful information was impeded in previous studies by either a lack of clinical samples or failure to validate the array results by other technologies. Moreover, the bacterial artificial chromosome array for identification of candidate genes remains problematic because several genes may be identified with a single probe.

The purpose of our study was to define the high-resolution regions of gains and losses in nasopharyngeal carcinoma, and through integration of copy number and gene expression profiles, to locate the putative oncogenes/tumor suppressor genes that may be involved in tumorigenesis. In addition, we also aimed to evaluate the changes of gene dosage associated with clinical cases. For that goal, we carried out a genome-wide survey of the consistent changes in DNA copy number and expression levels throughout the genomes of nasopharyngeal carcinoma cell lines, using array-based CGH and expression profiling experiments. Instead of a bacterial artificial chromosome array, the use of oligonucleotide microarray (average spatial resolution approximately 7 kb with multiple probes for each gene) for array CGH has the potential to indicate the nasopharyngeal carcinoma–associated genes directly. To evaluate the clinical prevalence, the most significant alterations found were validated in tissue microarrays using gene-specific fluorescence in situ hybridization (FISH). Sequentially we also examined the recurrent copy-number changes associated with patients’ survival rate. The results may provide a starting point for conducting further investigations by characterizing the roles of possible target genes found in this study.

Materials and Methods

Cell Lines. Three established human nasopharyngeal cell lines were investigated in this study. NPC-TW01 was obtained from a moderately differentiated nasopharyngeal carcinoma specimen, keratinizing squamous cell carcinoma (25). HONE1 was derived from poorly differentiated squamous carcinoma of the nasopharynx (26). NP460hTert is a telomerase-immortalized nasopharyngeal epithelial cell line with an almost diploid karyotype (27). All cells were cultured as described previously.

DNA and RNA Extraction. Genomic DNA was extracted using the DNeasy tissue kit (Qiagen) according to the manufacturer’s instructions, as described previously (12, 28). Total RNAs were extracted with an RNAeasy mini kit (Qiagen) according to the manufacturer’s instruction. The integrity of the DNA and RNA samples was verified using the RNA 6000 Nano Assay (Agilent Technologies).

Array-Based CGH Analysis. A microarray containing >244,000 oligonucleotide probes designed to cover coding, noncoding, and intergenic sequences in the human genome (Human Genome CGH microarray 244A, Agilent Technologies) was used for array-CGH analysis. Genomic DNA fragmentation, labeling, and array hybridization were done according to the standard array CGH protocol (version 4) provided by Agilent Technologies as described previously (12, 28). For each array-CGH experiment, we used DNA isolated from nasopharyngeal carcinoma cell lines as the experimental genome (cyanine 5–labeled) and the commercial healthy Caucasian male genome (Promega) as the reference (cyanine 3–labeled).

The hybridized arrays were scanned immediately at a resolution of 5 μ using an Agilent G2565BA DNA microarray scanner. The microarray images were analyzed using Agilent Feature Extraction software, version 8.1.1. Another custom analytical software package, Agilent CGH Analytics, version 3.4, was used for the subsequent data analysis. The locations of the copy number aberrations were calculated using the Aberration Detection Method 2 (ADM2) statistical algorithm. The ADM2 threshold was set at 9.0 to make an amplification or deletion call. According to these settings, the aberration score was generated automatically for each altered locus.

Gene Expression Microarray Analysis. The whole human genome oligo microarray kit (Agilent Technologies) and Agilent’s low RNA input linear amplification/one-color labeling kit were used for gene expression microarray analysis, all of which were conducted in biological duplicate. Target preparation and array hybridization essentially followed the protocol recommended by Agilent Technologies. Briefly, 1,000 ng of total RNA were reverse transcribed into cDNA using the T7 promoter primer. Cyanine 3-dCTP–labeled cRNA was synthesized from the cDNA by using the T7 RNA polymerase. To remove unincorporated nucleotides, the labeled cRNA was purified using an RNAeasy mini kit (Qiagen). Array hybridization was done in 100 μL of a hybridization mixture containing cRNA probes at 65°C for 17 h. The hybridized arrays were then washed and scanned using an Agilent G2565BA DNA microarray scanner. Fluorescence intensities of spots were quantified, background-subtracted, and dye-normalized by Feature Extraction software (Agilent Technologies). Raw data were imported and analyzed using GeneSpring GX (Agilent Technologies) to generate gene lists of differentially expressed (change in expression with fold change >2).

Quantitative Real-Time PCR. We designed PCR primers for the potential candidate genes within the defined aberration regions using the primer 3 program (http://www.genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi; Supplementary Table 1). Quantitative real time PCR was done on the cDNA extracted from NPC-TW01 and HONE1 cells. The cDNA from NP460hTert cells was used as the normal control for comparison. PCR reactions were done in triplicate and threshold cycle numbers were calculated using the iCycler software v2.3 (Bio-Rad Laboratories). Expression levels of candidate genes within each sample were determined by normalization to the expression level of GAPDH.
Tissue Microarray for FISH. We arranged 48 formalin-fixed, paraffin-embedded metastatic nasopharyngeal carcinoma tissues and two normal oral epithelia onto tissue microarrays to facilitate FISH. Four representative cores from each tumor were placed on the tissue microarrays. Acquisitions of tissue specimens and patient information were approved for this study by the Institutional Review Board at Taiwan University Hospital. Bacterial artificial chromosome clones, RP11-257J13 for ADAMTS9, CTD-2111L7 for LRIG1, CTD-3181O10 for GPR160, CTD-2320D24 for SKIL, RP11-806B4 for MPDZ, and RP11-909I1 for ADFP, were purchased from Invitrogen. Bacterial artificial chromosome clone RP11-61K12 was utilized as the universal control probe for all the genes tested because no copy number change was found within this region based on our array CGH data. The method used for FISH has been described in a previous study (12). Two individual scientists who were not aware of the tumor grade and clinical information evaluated FISH signals. Approximately 100 tumor cells were examined from each specimen. Gene amplification was defined as a ratio of gene probe signal to control probe signal >2.8. Gene deletion was defined as a negative ratio of control probe signal to gene probe signal <2.0.

Statistical Analysis and Clinical Correlation. The clinical information of nasopharyngeal carcinoma samples studied in this study was collected from clinical records, including tumor site, clinical stage, treatment history, recurrent status, and survivorship. Overall survival time was defined as the number of months between diagnosis and death or between diagnosis and the most recent follow-up. All calculations and statistical analyses were carried out using the SAS/STAT software package (SAS Institute) and plotted as survival curves using the Kaplan-Meier method. With this method, P values were evaluated by log-rank test.

Results

Impact of Copy Number Alterations on Gene Expression. Global copy number aberrations were analyzed using high-resolution oligoarray CGH on two nasopharyngeal carcinoma cell lines, NPC-TW01 and HONE1, which were originally defined as metastatic nasopharyngeal carcinoma. The overviews of array CGH profiles revealed high similarity between the nasopharyngeal carcinoma cell lines (Fig. 1 and Supplementary Table S2), but the degree of copy-number alterations was more severe in NPC-TW01 than in HONE1. There were 1,204 and 1,513 genes with copy-number gain (copy number variations with aberration score >0.5 and number of contiguous probes ≥3) in NPC-TW01 and HONE1, respectively. Among them, 850 were amplified in both cell lines (Gain-TH). There were 3,525 genes and 926 genes.

Figure 1. Whole genomic copy-number profiles of nasopharyngeal carcinoma cell lines TW01 and HONE1. The array CGH profile is shown adjacent to the ideogram of each chromosome. The results show the log2 value of the normalized signal intensity ratio from the array experiment. A log2 signal ratio of zero represents no aberrant copy number in TW01 and HONE1. Where log2 >0, it suggests amplified DNA copy-number for TW01/HONE1 relative to the reference human male genome. Where log2 <0, the reverse is inferred. Images were produced using CGH Analytics version 3.5.14 software. The array CGH data discussed in this publication have been deposited in the National Center for Biotechnology Information’s Gene Expression Omnibus (50) and are accessible through GEO series accession number GSE15172 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE15172).
with copy-number loss (copy number variations with aberration score <0.5 and number of contiguous probe ≥3) in NPC-TW01 and HONE1, respectively. Among them, 582 were deleted in both cell lines (Loss-TH). The most prominent CNVs (copy number variations) observed included gain on 3q26.2-q26.31, and loss on 3p21.2-q12.1, 9p21.3-p24.3, and nearly the whole Y chromosome (Supplementary Table S2). Interestingly, three of these four prominent CNVs, namely, gain on 3q26.2-q26.31, and loss on 3p21.2-q12.1 and 9p21.3-p24.3, have been identified as “hot spots” in a meta-analysis study that used CGH/array CGH data sets obtained from 188 nasopharyngeal carcinoma tumors (27). In an attempt to know the impact of ethnicity, a DNA pool from 10 healthy Taiwanese males was compared with the commercial Caucasian genome used in this current study. Our results showed that some microdeletions and microamplifications could be detected, but no prominent CNVs were found (Supplementary Fig. S1), suggesting the defined altered regions are related to nasopharyngeal carcinoma development.

The genome-wide effect of copy-number aberrations on transcription was evaluated by parallel conduct of gene expression microarray experiments on NPC-TW01 and HONE1 cells. In order to filter out differential genes unrelated to carcinogenesis of nasopharyngeal carcinoma, a gene expression database for premalignant nasopharyngeal epithelial cells was obtained from NP460hTert cells, and we used the commercial universal human RNA (Stratagene) as common reference. As shown in Venn diagrams in Supplementary Fig. S1A and B, a total of 1,272 genes were up-regulated (Up-TH) and 1,810 genes were down-regulated (Down-TH) in both cell lines. To identify the genes that were altered consistently in DNA copy number and expression levels, the comparisons of Gain-TH vs. Up-TH, and Loss-TH vs. Down-TH were made. We found that 12.4% (105/850) of Gain-TH genes were overexpressed and 15.1% (88/582) of Loss-TH genes were down-regulated. Interestingly, the 3q26.2-q26.32 amplicon contains 14 genes showing both dosage amplified and overexpressed, and each of the deletion regions 3p12.3-p14.2 and 9p21.3-p23 comprises eight down-regulated genes, respectively. The array CGH profiles for chromosomes 3 and 9 and the altered genes located in the prominent gain/loss regions are illustrated in Fig. 2. Notably, the striking deletion region at 3p14.2-p14.3 was not considered a candidate because the expression level of FHIT, the only gene involved in this region, did not correlate with genomic content. A high-density distribution of altered expressed genes emphasized the biological significance of changes in copy-number on 3p12.3-p14.2, 3q26.2-q26.32, and 9p21.3-p23. We also noted that a large number of the altered genes were cancer-related.

To validate mRNA expression levels detected by the expression arrays, all of the commonly dysregulated genes mapped to the three prominent copy number alterations were subjected to quantitative real-time PCR. Up to 87.5% to 92.9% of genes were cross-validated by both methods, as summarized in Fig. 3. Six genes were selected as the representative genes (two genes for each region) for further study because they showed higher fold changes as compared with the neighboring genes by qPCR validation. These genes were ADAMTS9 and LRIG1 located at 3p12.3-p14.2, GPR160 and SKIL at 3q26.2-q26.32, and ADFP and MPDZ at 9p21.3-p23.

**Genetic Alterations and Clinical Association.** To confirm the genetic alterations, dual-color FISH was done to...
detect the defined genetic altered regions in metastatic nasopharyngeal carcinoma tissues in an independent panel of 48 clinical samples. For the 3p12.3-p14.2 deletion region, homozygous deletion (FISH ratio < -2) of ADAMTS9 (red FISH signal) and LRIG1 (green signal) could be found in 13 (33%) and 12 (30%) of 40 nasopharyngeal carcinoma samples respectively (Fig. 4A and B, right). Using the same FISH probe sets, gene loci of ADAMTS9 and LRIG1 could be detected in normal or non-deleted nasopharyngeal carcinoma tissues (Supplementary Fig. S3A). Small genetic gain of the 3q26.2-q26.32 region (FISH ratios between 1.0 and 2.8) could be found in most of the tumor tissues based on the FISH analyses of GPR160 and SKIL. Moderate to high amplifications (FISH ratios > 2.8) were verified in 10 of 40 (25%) samples for GPR160 (green signals versus red signals) and in 13 of 40 (33%) samples for SKIL (red versus green) (Fig. 4A, middle). Similar to the 3p deletion, loss of heterozygosity of ADFFP (green versus red) and MPDZ (green versus red) at 9p21.3-p23 could be found in 6 of 40 (15%) nasopharyngeal carcinoma tumors (Fig. 4A, right). FISH analyses on the 3q nonamplified and 9p undeleted tumors are shown in Supplementary Fig. S3B and C.

To determine whether these genetic alterations affect the overall survival, Kaplan-Meier survival analysis was done to evaluate the clinical correlation. In this study, a more stringent criterion was used for subgrouping. Because there were two candidate genes in each altered genetic region, cases with both genes amplified or deleted were defined as abnormal and the rest as normal. Based on this definition, a significant association was found between 3p12.3-p14.2 deletion and poor clinical outcome (P = 0.0012; Fig. 5A). A similar finding also was found for the 3q26.2-q26.32 amplion (Fig. 5B).

Our data indicated that, using the approach of combined analyses between genetic alterations and gene expression profiles, we successfully defined the 3p deletion (3p12.3-p14.2) and 3q amplification (3q26.2-q26.32), and 9p21.3-p23 deletion are common genetic events during nasopharyngeal carcinoma development. We identified these altered regions in two well-known nasopharyngeal carcinoma cell lines, NPC-TW01 and HONE1, and later validated the findings by dual-color FISH analysis on a larger scale using paraffin-embedded metastatic nasopharyngeal carcinoma tissues (n = 48). The potential tumor suppressor genes, ADAMTS9 and LRIG1, and the suspected oncogenes, GPR160 and SKIL, were defined by combined analyses to indicate consistent biological relevance from genomic copy alterations to mRNA expression and clinical association. This study provides cogent evidence to suggest prognostic potentials of the defined 3p deletion (3p12.3-p14.2) and 3q amplification (3q26.2-q26.32) for clinical application.

Nasopharyngeal carcinoma arises from the epithelial cells that cover the surface and line the nasopharynx and typically metastasize to cervical lymph nodes for the advanced tumors. Because nasopharyngeal carcinoma is markedly radiosensitive, radiotherapy is the primary mode of treatment (29, 30). However, there is a high failure rate for such treatment once the tumor spreads to other organs and becomes metastatic (31). Identification of prognostic indicators that correlate with the clinical outcome would be helpful to develop new treatments for the severe types of nasopharyngeal carcinoma. For such clinical limitation, we focused particularly in this study on identifying novel genetic markers in metastatic tumors. The biological roles of newly defined candidate genes during nasopharyngeal carcinoma development are under investigation.

Gene amplification accounts for genomic instabilities in solid tumors (32). It has been proposed that activation of proto-oncogenes by amplification plays an important role in the development of many human cancers. Therefore, detection of specific gene amplifications in cancer cells can lead to the identification of putative oncogenes involved in growth control and metastasis. Our results confirm the previous conclusion that 3q26 is the principal amplified region in nasopharyngeal carcinoma genomes (17, 22-24, 33). Taking advantage of high-resolution array platforms, we have narrowed down the amplified region to 3q26.2-q26.31 (around 3.5 Mb) in two nasopharyngeal carcinoma cell lines and pinpointed several target genes within these defined regions. Among these genes, 13 also

![Figure 3](image_url)
have been found overexpressed by gene expression microarray and qPCR experiments. Consistent with these results, FISH analysis on TMA slides for SKIL (SKI-like oncogenes, 3q26) and GPR160 (G protein-coupled receptor 160, 3q26.2-q27) revealed a high incidence of amplification of these two genes in malignant nasopharyngeal carcinoma tumors. In particular, the occurrence of amplification of SKIL was up to 33% of the samples tested. SKIL, also known as SnoN, is a member of the Ski family of proto-oncogenes. Elevated SKIL expression has been observed in many human cancer types, including lung, stomach, breast, ovary, and thyroid (34-36). The role of SKIL in tumorigenesis is important but complex. Using human lung cancer and breast cancer cell lines, Zhu et al. showed that SKIL promotes mitogenic transformation at the early stages of tumorigenesis while inhibiting epithelial-to-mesenchymal transdifferentiation at later stages of tumor development (37). Further study is needed to reveal the roles SKIL plays in nasopharyngeal carcinoma and the functions of other genes mapped onto the 3q amplification region.

GPR160 was defined as a member of the G protein-coupled receptor family (38, 39). Although known to be a signaling molecule, the binding ligands and cellular functions of this gene in cancer development are largely unknown. Up-regulation of GPR160 has only been reported in prostate cancer (40) and our study provides the first evidence of involvement of GPR160 in nasopharyngeal carcinoma development and progression.

Of note, the prominent amplification region 3q26.2-q26.31 detected in this study is precisely coincident with one of the amplification regions observed in nasopharyngeal carcinoma cells harboring recurrent EBV reactivations (12). This finding not only strengthens the involvement of EBV in the pathogenesis of nasopharyngeal carcinoma, but also provides supporting evidence for our hypothesis that frequent EBV reactivation may enhance genome instability, in nonrandomly distribution patterns, and accelerate the progression of nasopharyngeal carcinoma (11, 12). On the other hand, these results suggest that 3q26.2-q26.31 also may be a critical region for nasopharyngeal carcinoma malignancy, and therefore overexpressed genes located in this region warrant further detailed analysis.

Loss of 3p and 9p are other genomic abnormalities that have been described frequently (13, 15, 41-44). Especially for 3p, this is the most commonly deleted region in nasopharyngeal carcinoma, as already known. Moreover, loss of 3p has been identified as an early event in the tumorigenesis of nasopharyngeal carcinoma (21). Again, we have refined the affected regions to 3p12.3-p14.2 and 9p21.3-p23, as well as pinpointed potential tumor

Figure 4. FISH analysis on nasopharyngeal carcinoma tissue microarray. A. The distribution of FISH signal ratio of nasopharyngeal carcinoma tissue samples and the detection rate is indicated under each FISH probe. The definition of FISH signal ratio for gene amplification or deletion is described in Materials and Methods. B. The representative FISH images for loss of ADAMTS9 (target gene versus control gene, red signal versus green signal), LRIG1 (green versus red), ADFP (green versus red), and MDPZ (green versus red), and amplification of GPR160 (green versus red) and SKIL (red versus green).
suppressor genes within these deleted regions by coupled analysis profiles of array CGH and gene expression array. There is a high density of down-regulated genes located in the deleted 3p12.3-p14.2 and significant association of these genomic deletion with lower survival rates. The findings highlight the importance of genes detected in 3p12.3-p14.2.

Among the genetic markers described here, both ADAMTS9 and LRIG have been reported to be putative tumor suppressor genes during cancer development (45-47). It is of particular note that gene silencing of ADAMTS9, mapping to 3p14.2-p14.3, was recently found associated with nasopharyngeal carcinoma and esophageal squamous cell carcinoma (45, 46). Lung et al. showed that ADAMTS9 expression was down-regulated or absent with a much higher frequency (up to 73.9%) in lymph node metastases of nasopharyngeal carcinoma specimens than those from primary tumors. At the same time, they also showed that down-regulation or loss of ADAMTS9 expression was a consequence of promoter hypermethylation. Consistent with these previous results, we found that the level of ADAMTS9 transcripts were reduced or lost completely in both nasopharyngeal carcinoma cell lines analyzed (Fig. 3), indicating the critical role of this gene in nasopharyngeal carcinoma. On the other hand, our data of array CGH and FISH showed loss of the ADAMTS9 locus, rather than promoter hypermethylation, in both HONE1 and NPC-TW01 nasopharyngeal carcinoma cell lines and in 30% of metastasis nasopharyngeal carcinoma tumors. These results may imply that inactivation of ADAMTS9 in metastatic nasopharyngeal carcinoma could be achieved by genetic deletion, as well as epigenetic modifications. In addition, the crucial suppressive role played by ADAMTS9 may have been overlooked in the past. Furthermore, our study also suggested deletion of LRIG as a cogenetic event during nasopharyngeal carcinoma development. Because molecular genetic changes in cancer cells are heritable traits after long-term clonal selection/expansion (48, 49), our approach of using the 3p genetic loss as a prognostic marker indicated a higher sensitivity to predict clinical outcome than ADAMTS9 immunohistochemistry staining. Although only six genes were verified in this study, other genes mapping to the defined amplified and deleted regions may still possess potential roles in cancer development based on the genome-transcriptome comparison. For example, ROBO1 and SKIL at 3q26.2-q26.31 were verified as important genes involved in EBV reactivation (11). Interaction or combination between different genes within the defined regions may provide survival advantages for cancer development/progression, and the molecular mechanism needs further investigation.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

References
Chromosome 3p12.3-p14.2 and 3q26.2-q26.32 Are Genomic Markers for Prognosis of Advanced Nasopharyngeal Carcinoma


Updated version
Access the most recent version of this article at:
http://cebp.aacrjournals.org/content/18/10/2709

Supplementary Material
Access the most recent supplemental material at:
http://cebp.aacrjournals.org/content/suppl/2009/10/01/1055-9965.EPI-09-0349.DC1

Cited articles
This article cites 50 articles, 12 of which you can access for free at:
http://cebp.aacrjournals.org/content/18/10/2709.full.html#ref-list-1

Citing articles
This article has been cited by 2 HighWire-hosted articles. Access the articles at:
/content/18/10/2709.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.