Array-Based Comparative Genomic Hybridization Analysis Identified Cyclin D1 as a Target Oncogene at 11q13.3 in Nasopharyngeal Carcinoma

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Abstract

Nasopharyngeal carcinoma is highly prevalent in Southern China and Southeast Asia. To unveil the molecular basis of this endemic disease, high-resolution comparative genomic hybridization arrays were used for systematic investigation of genomic abnormalities in 26 nasopharyngeal carcinoma samples. A comprehensive picture of genetic lesions associated with tumorigenesis of nasopharyngeal carcinoma was generated. Consistent chromosomal gains were frequently found on 1q, 3q, 8q, 11q, 12p, and 12q. High incidences of nonrandom losses were identified on chromosomes 3p, 9p, 11q, 14q, and 16q. In addition to previously characterized regions, we have identified several novel minimal regions of gains, including 3q27.3-28, 8q21-24, 11q13.1-13.3, and 12q13, which may harbor candidate nasopharyngeal carcinoma–associated oncogenes. In this study, gain of 11q13.1-13.3 was the most frequently detected chromosomal aberration and a 5.3-Mb amplicon was delineated at this region. Within this 11q13 amplicon, concordant amplification and overexpression of cyclin D1 (CCND1) oncogene was found in nasopharyngeal carcinoma cell lines, xenografts, and primary tumors. Knockdown of cyclin D1 by small interfering RNA in nasopharyngeal carcinoma cell lines led to significant decrease of cell proliferation. The findings suggest that cyclin D1 is a target oncogene at 11q13 in nasopharyngeal carcinoma and its activation plays a significant role in nasopharyngeal carcinoma tumorigenesis. (Cancer Res 2005; 65(18): 8125-33)

Introduction

Nasopharyngeal carcinoma is a common cancer in Southern China and Southeast Asia but is rare in Western countries. Radiotherapy is an effective treatment for this cancer and >80% of the patients treated with early diseases are long-term survivors. The present problem lies in nasopharyngeal carcinoma patients diagnosed at late stage, where treatment is much less effective and difficult. To derive targets for novel therapeutic strategies, unveiling the molecular basis of this cancer is important for control of this endemic disease (1, 2).

To investigate the genetic alterations involved in nasopharyngeal carcinoma tumorigenesis, we have done systematic studies to search for tumor suppressor loci in nasopharyngeal carcinoma by high-resolution allelotyping and comparative genomic hybridization (CGH) analysis. Frequent allelic deletions were revealed on chromosomes 3p, 9p, 9q, 11q, 13q, 14q, and 16q (3–8). Multiple minimal deletion regions delineated the nasopharyngeal carcinoma-associated tumor suppressor loci to 3p14-24.2, 9p21.3, 11q12-23, 13q12-14, 13q31-32, 14q24-32, and 16q22-23, whereas a homozygous deletion region was located at 9p21.3 (3, 5–8). Those findings led to identification of critical tumor suppressor genes (p16, RASSF1A, and TSLC1), which involved in nasopharyngeal carcinoma development (8–10). Nevertheless, information of oncogene(s) activated in nasopharyngeal carcinoma is rare. Our previous molecular cytogenetic studies have identified regions with frequent gain of genetic materials on chromosomes 1q, 3q, 7, 8, 9q, 12q, and 20q (11, 12). In this study, we used high-density genomic array chips to characterize the chromosomal aberrations in nasopharyngeal carcinoma genome comprehensively. The array chips contain 1,803 BAC clones that spanned the human genome at about 1 to 3Mb resolution. A series of 26 nasopharyngeal carcinoma samples were investigated. These included two nasopharyngeal carcinoma xenografts, three nasopharyngeal carcinoma cell lines, and 21 microdissected primary tumors. The results of our study generate a detailed map of the multiple genetic lesions involved in nasopharyngeal carcinoma tumorigenesis. Furthermore, we have characterized the most commonly detected amplicon in the nasopharyngeal carcinoma genome at 11q13.3 for identification of target nasopharyngeal carcinoma–associated oncogene(s).

Materials and Methods

Cell lines and xenografts. Three well-characterized nasopharyngeal carcinoma cell lines (C666-1, HK-1, and HONE-1) and two nasopharyngeal carcinoma xenografts (xeno-NPC8 and xeno-2117) were included in this study (13–15). The cell lines were cultured with RPMI 1640 supplemented with 10% fetal bovine serum, 1% t-glutamine, and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA).
Primary tumors. For array CGH analysis, all of the 21 nasopharyngeal carcinoma biopsies were obtained from patients with informed consent prior treatment from the Prince of Wales Hospital and Queen Mary Hospital. The biopsies were embedded in ornithine carbamyl transferase compound and stored at −80°C until retrieval for DNA extraction. Each sample was histologically examined and shown to contain at least 70% of tumor cells. To increase content of neoplastic cells, microdissection was done on some of the samples. A panel of 43 archival specimens was included in immunohistochemistry and fluorescence in situ hybridization (FISH) analysis. The samples were collected from the Pathology Tissue Bank in the Department of Anatomical and Cellular Pathology at the Prince of Wales Hospital.

Array comparative genomic hybridization analysis. The BAC arrays used in the current study were obtained from Prof. JW. Gray at the University of California San Francisco Cancer Center. Each microarray contained 1,803 clones distributed throughout the human genome. A detailed list of the BAC clones used was shown in supplementary data (Supplement 1). Array CGH analysis was done as described previously (16–20). Briefly, tumor DNAs and reference DNAs were labeled by random priming (Invitrogen) with Cy3-dUTP and Cy5-dUTP (Amersham Pharmacia, Piscataway, NJ), respectively. Five hundred nanograms of each of the labeled tumor and reference DNAs were cohybridized to microarrays in the presence of tRNA and human Cot-1 DNA (Invitrogen Life Technologies). After hybridization for 2 days, post-hybridization wash was done and the fluorescence single-color intensity images of Cy3 and Cy5 microarray images were captured using GenePix Pro 4.0 (Axon Instruments, Inc., Foster City, CA). The images were analyzed by Spot and Sproc software as described previously (18, 19). The software automatically identifies targets on the array and calculates Cy3 and Cy5 intensities for all targets. The Cy3/Cy5 ratios indicate the degree of gain or loss of copy number.

Fluorescence in situ hybridization. FISH analysis was done to confirm copy number changes of 9p21.3, 11q13.3, and 12p12-13 identified in the array CGH study. A commercial dual color FISH probe of 9p21.3 and chromosome 9 centromere was used to evaluate 9p21.3 loss (LS0p21/CEP9 Vysis). Study of 11q13.3 amplification used the clone RP11-3006f6 as a test probe and a commercially available chromosome 11 α-satellite as reference probe. Two BAC clones, RP11-877E17 (12p12.1) and RP11-451H11 (12p13.31), were used for investigation of 12p12-13 gain with chromosome 12 α-satellite as a reference probe. Dual-color FISH analysis was conducted on 43 primary nasopharyngeal carcinoma, xeno-2117, xeno-NPC8, and metaphase spreads of cell line HK-1. In each case, at least 100 cells were analyzed. Amplifications and gains were scored when ratios of test to reference probes were >2 and 1.2, respectively. Samples with signal ratios of test to reference probes of <0.8 were counted as deletion cases.

Investigation of copy number changes of chromosome 11q13.3 locus of oncogene. High level amplification of 11q13.3 was detected on nasopharyngeal carcinoma cell line HK-1, xeno-2117, and xeno-NPC8. For rapid investigation of copy number changes of common oncogenes located at 11q13, commercial GenoSensor 300 microarray chips were used to investigate the nasopharyngeal carcinoma–associated oncogenes. The chips contained 287 target common cancer genes with six of them are known oncogenes at 11q13 region. These were MEC, CCND1, FGFR3, EMD, GAR1, and PAK1. The gene-specific array CGH was done as described previously (11).

Real-time quantitative reverse transcription-PCR. Expression levels of the aforementioned six candidate oncogenes at 11q13 were evaluated by quantitative reverse transcription-PCR (RT-PCR) as described (21). Total RNAs of a normal epithelial outgrowth was included as a control. The expression levels of the housekeeping gene β-actin of each sample were determined for normalization. Results of the fold difference (N) in expression levels of genes relative to β-actin gene was calculated using the formula N = 2Δ C t sample. The ΔC t value of each sample was determined by subtracting the average C t value of genes from the average C t value of the β-actin gene. Expression levels of each sample were normalized, with the mean N value in the normal nasopharyngeal epithelial outgrowths would equal to 1.

Western blotting and immunohistochemistry analysis. The expression of cyclin D1 in nasopharyngeal carcinoma cell lines and xenografts was investigated by Western blotting. A normal nasopharyngeal epithelial outgrowth was included as a control. Proteins were extracted from the samples for Western blot analysis using anti-cyclin D1 monoclonal antibody (Lab Vision, Fremont, CA) and polyclonal antibody of actin (Santa Cruz Biotechnology, Santa Cruz, CA). To investigate the expression levels of p16 and cyclin D1 in primary nasopharyngeal carcinoma, immunohistochemistry analysis of the 38 formalin-fixed, paraffin embedded sections of nasopharyngeal carcinoma cases were conducted by using the anti-cyclin D1 monoclonal antibody (Lab Vision) or antibody of p16-IND4a monoclonal antibody (Biocare Medical, Concord, CA).

Small interfering RNA–mediated suppression of CCND1 gene in nasopharyngeal carcinoma cells. Effect of CCND1 on nasopharyngeal carcinoma cell growth was further investigated by small interference RNA (siRNA)–mediated suppression. Two nasopharyngeal carcinoma cell lines, HK-1 and C666-1, were transfected with siRNA targeted against CCND1 gene (Invitrogen). By using LipofectAMINE 2000 and Opti-MEME I-reduced serum medium (Invitrogen). A nonspecific siRNA oligo with same level of GC content was included as a control (Invitrogen, San Diego, CA). Efficiency of siRNA mediated suppression of CCND1 gene in nasopharyngeal carcinoma cells was determined by measuring RNA and protein expression of CCND1 on the transfected cells. Cell proliferation analysis of the nasopharyngeal carcinoma cells was examined by staining cells with trypan blue and counted with a hemocytometer (Sigma, St. Louis, MO).

Results

Genomic profiles of nasopharyngeal carcinoma cell lines, xenografts, and primary tumors. We have done a total of five normal versus normal hybridizations to define the normal variations of the test to reference (Cy3/Cy5) intensity ratio for each target clone. Our normal male-to-female hybridizations showed that ratios of green to red signals were centered at 0 for the autosomes (Fig. 1A). With reference to previous array CGH studies, thresholds for copy number gain and loss were log2 ratio 0.2 and −0.2, respectively (16, 17, 19, 20). Nonrandom (detected in more than two cases) homozygous deletion and high level amplifications were scored when log2 ratio was less than −1 or >1, respectively. Representative array CGH profiles of nasopharyngeal carcinoma cell line, xenograft, and primary tumor were shown on Fig. 1B–D. Multiple genetic aberrations including chromosomal gains and losses were detected on all tumor samples. Figure 2A summarized genome-wide frequency of copy number alterations for the 26 nasopharyngeal carcinoma samples. Percentages of chromosomal gains or losses of each clone were indicated in top and bottom of the figure, respectively. As reported in previous CGH analysis, chromosomal losses were more frequently detected than gains. The results from array-based CGH analysis have delineated multiple minimal deletion regions and amplicons. Figure 2B–D illustrated representative CGH profiles of some common chromosomal aberrations. Nonrandom chromosomal regions of gains and losses were defined when genetic imbalances detected in >50% cases of more than five consecutive clones (20).

Table 1 shows detail information of the nonrandom chromosomal aberrations. Concordant high incidences of chromosomal aberrations were loss of 3p21.25 (88.5%), 9p21.3-2p43.2 (76.9%), 11q12-qter (73.4%), 14q11.2-qter (73.1%), and 16q21-qter (57.7 %) and gain of 1q24.3-32.1 (53.8%), 1q42-ter (50%), 3p27.3-28 (50%), 8q21-24 (53.8%), 11q13.1-13.3 (61.5%), 12p12.1-13.3 (57.7%), and 12q13.13-13.2 (53.8%). Sizes of the corresponding regions of chromosomal imbalances ranged from 4.04 to 91.08 Mb (Table 1).

We have compared the genetic profiles between the 21 primary nasopharyngeal carcinomas and the five cell lines/xenografts. Most of the aforementioned chromosomal aberrations were
consistently detected in both primary tumors and cell lines/xenografts (Table 1). Similar incidences of copy number changes on these regions were showed between two groups of samples. Nevertheless, several aberrations included frequent losses of 1p13-p31, 4q31, 6p, 8p11, 12q24, 18p11-q23, and 22q12-13 were only identified in cell lines and xenografts. Clones with highest incidences of gain or loss and those with high-level amplification and homozygous deletion were listed in supplementary tables.

Figure 1. Profiles of array-based comparative genomic hybridization analysis. The vertical lines indicate separation of chromosomes. Log2 ratios of the 1,803 clones were plotted based on chromosome position. The clones ordered from the 1p telomere on the left to the Xq telomere on the right. A, profile of a normal male and female hybridization. Copy loss was illustrated on chromosome X, whereas no genetic imbalances were detected on the autosomes. B-D, representative array CGH profiles of a primary nasopharyngeal carcinoma (NPC-4), a xenograft (xeno-2117), and a cell line (C666-1).
Figure 2. A, summary of array-based comparative genomic hybridization copy number profiles of 26 nasopharyngeal carcinoma cases. Clones are ordered from chromosomes 1 to 22 and within each chromosome on the basis of the UCSC mapping position (http://genome.ucsc.edu/). Incidences of gains (top) and losses (bottom) of the BACs and the affected chromosomal regions. B, representative profiles of loss of 3p in xeno-2117 (log2 ratio = 0.5) and homozygous deletion of 9p21 (log2 ratio < -1) in primary tumor NPC-4. C, illustration of 12p amplification (log2 ratio = 2) in xeno-2117 and chromosomal gain in NPC-4 (log2 ratio > 0.5). D, NPC-4 was found to have gains at 11q13 and 11q21 (log2 ratio > 0.5), whereas log2 ratio of other clones at 11q were around -0.5 that indicated copy loss of this region. NPC-4 was found to have 14q loss with a log2 ratio of -0.5 at the clones.
High-level amplification was commonly found in 1p21.1, 3q26.2-26.31, 11p11.2, 11q13.3, and 12p12.1-13.2. These regions located several oncogenes including EVI1, EIF5A2, PLD1, CCND1, and TEL. Homozygous deletions were detected in 1q44, 3p21, 3p14.1, 9p21.3, 11q21, 11q25, 16p11.2, 16q24.3, 17p13.3, 18p11.21, and 18q23 (Supplementary Table 1). The most frequently gained clones are RP11-300I6 at 11q13.3, RP11-451H11 at 1q44, and RP11-418C2 at 12p13.2 (Supplementary Table 2). On the other hand, RP5-84708 at 1q42.2 and RP11-589M4 at 1q42.21 are the most frequently lost clones. Almost all of these clones were located within the defined nonrandom chromosomal regions of gains and losses. The findings strongly suggested that these regions of chromosomal imbalances harbor nasopharyngeal carcinoma-associated tumor suppressor genes and oncogenes.

In the present study, 11q13.1-13.3 is the region with most frequent detection of gain. We are the first group to delineate a 5.3-Mb amplification of 11q13.3 by FISH analysis. Two double-hit amplifications were detected in xeno-2117 and 12p12.1-13.2. These regions located several oncogenes including EVI1, EIF5A2, PLD1, CCND1, and TEL. Concordantly, amplification of two clones RP5-940J5 (12p13.31) and RP11-418C2 (12p12-13.2) were detected in two cases, whereas copy number gain were found in 16 of 26 cases (61.5%; Table 1). High incidence of 12p11-13 gain was further verified with FISH analysis in 39 additional primary nasopharyngeal carcinomas by using two probes on 12p12.1 (RP11-877E17) and 12p13.11 (RP11-451H11). Both of the 12p12.1 (RP11-877E17) and 12p13.11 (RP11-451H11) located clones were found to have copy number gain in 22 of 43 samples (51.1%). Twenty-seven of the cases (62.8%) showed gain of either one of these two clones.

Among the multiple regions of loss, we have delineated a novel minimal deletion region at 16q21-23.2. The region is 15.85 Mb that flanked by RP11-22903 and RP11-18F14. In addition to copy loss, homozygous deletion was detected at two overlapped BACs (RP11-79A1 and RP11-368I7) located at 16q24.3 (Supplementary Table 1). These two clones covered a region of 0.21 Mb that located several candidates genes including cancer-related genes CDK10 and FANCA. Concordant to previous findings, both frequent loss of 9p (76.9%) and homozygous deletions of 9p21.3 (two cases) were detected. Because the well-known nasopharyngeal carcinoma–associated tumor suppressor gene p16 is located in this region, we also validated the deletions of p16 locus by FISH (Fig. 3C-D). A representative homozygous deletion of 9p21.3 in a primary tumor was shown in Fig. 3D. To further confirm the array-based CGH findings, we have examined the frequent deletion of 9p21.3 in an independent series of primary nasopharyngeal carcinomas. Concordant high incidence of deletion of 9p21.3 was found in 23 of 43 cases (53.5%).

**Amplification and overexpression of common oncogenes at 11q13.** Results of high-resolution array CGH analysis revealed that increased copy number of 11q13 is frequently detected in nasopharyngeal carcinoma. We applied commercial GenoSensor 300 microarray chips to further investigate copy number changes of six common oncogenes located at chromosomes 11q13. These include MEG1, CCND1, FGFR3, EMSI, GARP, and PAK1 genes. Nasopharyngeal carcinoma cell lines HK-1, xeno-2117, and xeno-NPC8, which with high level amplifications at these regions, were

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**Table 1.** Nonrandom chromosomal regions of gains and losses identified in 26 nasopharyngeal carcinoma samples by array-based CGH analysis.

<table>
<thead>
<tr>
<th>Regions of gains</th>
<th>Flanking BAC clones</th>
<th>Length of amplicon (Mb)</th>
<th>Total incidence (%)</th>
<th>Incidence in cell lines/xenografts (%)</th>
<th>Incidence in primary tumors (%)</th>
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<tr>
<td>1q24.3-32.1</td>
<td>RP11-105D12, RP11-15D115</td>
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<td>1q21-qter</td>
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<td>80</td>
<td>47.6</td>
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<td>8q21-24</td>
<td>RP11-155H18, RP11-94H5</td>
<td>5.3</td>
<td>61.5</td>
<td>60</td>
<td>61.9</td>
</tr>
<tr>
<td>11q13.1-13.3</td>
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<td>12p12.1-13.3</td>
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<td>53.8</td>
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<table>
<thead>
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<th>Regions of losses</th>
<th>Flanking BAC clones</th>
<th>Length of deletion region (Mb)</th>
<th>Total incidence (%)</th>
<th>Incidence in cell lines/xenografts (%)</th>
<th>Incidence in primary tumors (%)</th>
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<td>9p21.3-p24.3</td>
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<td>80.8</td>
<td>60</td>
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<td>14q11.2-qter</td>
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<td>16q21-qter</td>
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<td>40</td>
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analyzed as described in our previous study. As shown in Fig. 4A, high-level amplifications were detected on four genes (CCND1, FGF3, EMS1, and PAK1) located at 11q13 in the nasopharyngeal carcinoma samples. Expression levels of the 11q13 genes were also shown in Fig. 4A. Overexpressions were scored with expression level higher than 2-fold of the normal epithelial outgrowth. Among these six genes, concordant high level amplification and overexpression were detected on CCND1 located at 11q13.3. Overexpression of cyclin D1 in the nasopharyngeal carcinoma cell lines and xenografts was also confirmed by Western blotting (Fig. 4B). By immunohistochemistry analysis, expressions of cyclin D1 in the nasopharyngeal carcinoma cell lines and xenografts was also confirmed by Western blotting (Fig. 4B). By immunohistochemistry analysis, expressions of cyclin D1 were investigated in 38 paraffin-embedded primary nasopharyngeal carcinomas in which copy number gain of 11q13 have been determined by FISH analysis (Fig. 5A). Cases with >20% of the cells that showed positive staining were scored to have overexpression of cyclin D1. Thirty-five of the 38 cases (92.1%) showed overexpression of cyclin D1. Concordant correlation of copy number gain of cyclin D1 and overexpression of the gene was observed in 27 cases which with both gain of 11q13 and overexpression of cyclin D1 (Fig. 5B). This finding suggested that cyclin D1 is the critical target oncogene for 11q13 amplicon in nasopharyngeal carcinoma.

Because of the functional association of cyclin D1 and p16, we have also examined the correlation of loss of p16 and activation of cyclin D1 in nasopharyngeal carcinoma samples (Fig. 5C). By immunohistochemistry, p16 expression has been investigated in the primary tumors. Cases with <20% of the cells that showed positive staining were scored to have reduced expression of p16. Loss of p16 was found in 33 of 38 primary cases (86.8%). In 31 of 38 primary tumors, both overexpression of cyclin D1 and loss of p16 expression were detected. The results suggested that alterations of both p16 and CCND1 are critical events for nasopharyngeal carcinoma development.

**Transfection of small interfering RNA against CCND1 in nasopharyngeal carcinoma cells.** To illustrate the oncogenic roles of CCND1 on nasopharyngeal carcinoma cells, cell lines HK-1 and C666-1 were transfected with siRNA targeted against CCND1 gene to knockdown its expression. The siRNA-treated cells were collected at 72 hours for quantitative RT-PCR and Western blot analysis. Both RNA and protein expressions of cyclin D1 on siRNA-treated HK-1 and C666-1 cells were significantly reduced when compared with LipoFectAMINE only and nonspecific siRNA-treated cells. The expression of cyclin D1 was reduced >90% in siRNA-treated HK-1 cells, as shown in Fig. 4C. Significant reduction of cell proliferation was found in 75% of the siRNA-treated HK-1 cells (Fig. 4D). Reduction of cell proliferation (>45%) was also detected on C666-1 cells (Supplementary Fig. 1). This finding indicated that cyclin D1 overexpression is necessary for the growth of the nasopharyngeal carcinoma cells.

**Discussion**

Our previous CGH findings identified a number of genetic aberrations involved in the initiation and progression of nasopharyngeal carcinoma (11, 12). To delineate the minimal regions of deletions and ampilcons in this cancer, we have carried a comprehensive investigation of genomic abnormalities involved in nasopharyngeal carcinoma progression by high-resolution array-based comparative genomic hybridization. The results provided a detailed picture of multiple genetic lesions in nasopharyngeal carcinoma genome and also give hints for further
Consistent with previous findings, we have found nonrandom losses on chromosomes 3p21-25, 9p21.3-p24.3, 11q12.2-qter, 14q11.2-qter, and 16q21-qter (3–5, 7, 8). High incidences of loss and homozygous deletion on 3p and 9p are concordant with our previous studies (4, 7). With a higher resolution in current array CGH template, we have also identified a novel minimal deletion region at 16q21-23.2 and homozygous deletions at 16q24.3. The finding suggests a novel tumor suppressor locus and provides important hints for identifying target genes involved in nasopharyngeal carcinoma development. We have also found recurrent chromosomal gains on multiple chromosomal regions. Novel amplics of nasopharyngeal carcinoma were identified on 11q13.1-13.3, 12p11-13.3, and 12q13.13-13.2. Among these amplics, 11q13.1-13.3 is most frequently detected. Oncogene(s) reside on this chromosomal region may play a critical role in the development of nasopharyngeal carcinoma. Identification of oncogenes located at these regions will help to delineate the genetics underlying the development of this disease. Current array

![Figure 4](image-url)

**Figure 4.** A, summary of copy number changes and expression level analysis results of common oncogenes located at 11q. Cases with amplification or overexpression were shaded. Genes with concordant high level amplifications and overexpressions were in bold. Six genes were located at 11q13.1-11q13.3. CCND1, EMS1, and PAK1 genes had concordant level amplifications and overexpressions in three, two, and one of the cases, respectively. No expression of FGFR3 and GARP were detected on the three nasopharyngeal carcinoma samples and normal epithelial outgrowth of nasopharynx. B, Western blot analysis of CCND1 in nasopharyngeal carcinoma cell lines and xenografts. Expression level of actin was included as a control. Overexpression of CCND1 was detected on all of the samples including cell lines C666-1, HK-1, and HONE-1 and xenografts xeno-NPC8, xeno-666, and xeno-2117. (Cell line C666-1 was derived from xeno-666, which was not included in the array CGH analysis due to the detection of identical chromosomal aberrations). C, illustration of knockdown of CCND1 gene in HK-1 cells by transfection of 40 nmol/L of siRNA targeted against CCND1 gene for 72 hours by Western blot analysis. More than 90% reduction of cyclin D1 expression in siRNA-treated HK-1 cells was detected when compared with controls of LipofectAMINE only and siRNA oligo with same level of GC content. D, 75% reduction of cell proliferation was found in siRNA-treated HK-1 cells.
functions including regulation of various transcription factors, being a cdk-dependent regulator of the cell cycle, cdk-independent important in the initiation of DNA replication (26). In addition to phosphorylate RB1 which lead to release of transcription factors adenovirus which resulted in cell transformation by complementing a defective growth advantage (24). Oncogenic function of which lead to lose of normal regulatory constraints and confer stimulates tumor development in corporate with other oncogenes and causes transformation to a malignant phenotype. It can as regulators of cyclin-dependent kinase (cdk) kinases. Over-protein belongs to the highly conserved cyclin family that functions is a protein derived from PRAD1 (parathyroid adenomatosis 1). The role of nasopharyngeal carcinoma cells. Our result showed the critical reduction of cell proliferation was found in siRNA-treated against nasopharyngeal carcinoma cells by transfection of siRNA targeted tumors. We have studied the oncogenic properties of nasopharyngeal carcinoma cell lines, xenografts, and primary located within this amplicon were consistently detected in CCND1–mediated oncogenesis (27). Our previous studies of nasopharyngeal carcinoma have shown the inactivation of p16 gene in all nasopharyngeal carcinoma cell lines and xenografts (5, 8). Importantly, either homozygous deletion or promoter hypermethylation of p16 gene were detected in majority of primary nasopharyngeal carcinomas (5, 8). Functional studies also showed that extropic expression of p16-induced G1 arrest in nasopharyngeal carcinoma cells (28). In the present study, we have also investigated the activation of cyclin D1 and loss of p16 in a same series of primary tumors. Concurrent overexpression of cyclin D1 and reduced expression of p16 was found in nasopharyngeal carcinoma. The findings suggested that both inactivation of p16 and activation of CCND1 may be important in alteration of cell cycle controls and development of nasopharyngeal carcinoma. Thus, cyclin D1 may be an important target for development of more effective therapeutic strategies of nasopharyngeal carcinoma. Inhibition of cyclin D1 by its inhibitor, such as acyclic retinoid and flavopiridol, might be a novel approach for the treatment of this cancer (29).

In conclusion, we have done a comprehensive whole genome analysis to decipher the nasopharyngeal carcinoma genome. This leads to discovery of multiple novel amplicons in nasopharyngeal carcinoma and shows that CCND1 is a nasopharyngeal carcinoma–associated oncogene.

CGH analysis fine mapped a 5.3-Mb amplicon core at 11q13.1-13.3. Concordant amplification and overexpression of CCND1 gene located within this amplicon were consistently detected in nasopharyngeal carcinoma cell lines, xenografts, and primary tumors. We have studied the oncogenic properties of CCND1 in nasopharyngeal carcinoma cells by transfection of siRNA targeted against CCND1 into nasopharyngeal carcinoma cells. A dramatic reduction of cell proliferation was found in siRNA-treated nasopharyngeal carcinoma cells. Our result showed the critical role of CCND1 gene in nasopharyngeal carcinoma cells. Hence, activation of CCND1 gene may contribute to the development of nasopharyngeal carcinoma. CCND1 gene was a common oncogene of human cancers such as breast cancer, head and neck squamous cell carcinomas, lymphomas, and gliomas (22, 23). Cyclin D1, the CCND1 encoding protein, is a protein derived from PRAD1 (parathyroid adenomatosis 1). The protein belongs to the highly conserved cyclin family that functions as regulators of cyclin-dependent kinase (cdk) kinases. Overexpression of cyclin D1 releases a cell from its normal controls and causes transformation to a malignant phenotype. It can stimulate tumor development in corporate with other oncogenes which lead to lose of normal regulatory constraints and confer growth advantage (24). Oncogenic function of CCND1 gene was illustrated by transsection of its full-length cDNA in cultured cells which resulted in cell transformation by complementing a defective adenovirus E1A oncogene (25). Overexpression of cyclins is critical in the regulation of cell cycle progression. Cyclin D1 controls cell cycle at the G1-S (start) transition by interact with CDK4 and CDK6 to phosphorylate RB1 which lead to release of transcription factors important in the initiation of DNA replication (26). In addition to being a cdk-dependent regulator of the cell cycle, cdk-independent functions including regulation of various transcription factors, histone acetylases and deacetylases, are reported to be important in cyclin D1–mediated oncogenesis (27). Our previous studies of nasopharyngeal carcinoma have shown the inactivation of p16 gene in all nasopharyngeal carcinoma cell lines and xenografts (5, 8). Importantly, either homozygous deletion or promoter hypermethylation of p16 gene were detected in majority of primary nasopharyngeal carcinomas (5, 8). Functional studies also showed that extropic expression of p16-induced G1 arrest in nasopharyngeal carcinoma cells (28). In the present study, we have also investigated the activation of cyclin D1 and loss of p16 in a same series of primary tumors. Concurrent overexpression of cyclin D1 and reduced expression of p16 was found in nasopharyngeal carcinoma. The findings suggested that both inactivation of p16 and activation of CCND1 may be important in alteration of cell cycle controls and development of nasopharyngeal carcinoma. Thus, cyclin D1 may be an important target for development of more effective therapeutic strategies of nasopharyngeal carcinoma. Inhibition of cyclin D1 by its inhibitor, such as acyclic retinoid and flavopiridol, might be a novel approach for the treatment of this cancer (29).

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Figure 5. Expression of cyclin D1 and p16 in primary nasopharyngeal carcinoma. A, top, immunohistochemical staining for Cyclin D1. Left, focal positivity of Cyclin D1, especially in the basal layer, is seen in the respiratory epithelium of nasopharynx (original magnification, ×400). Middle, a case of nasopharyngeal carcinoma shows low level of expression with occasional scattered carcinoma cells exhibiting positive staining for Cyclin D1 (original magnification, ×400). Right, diffuse and strong nuclear staining for Cyclin D1 is shown in this case of nasopharyngeal carcinoma (original magnification, ×400). Bottom, immunohistochemical staining for p16. Left, p16 expression in the normal respiratory epithelium of nasopharynx of a representative nasopharyngeal carcinoma case. This case has focal staining for p16, which tend to be positive in the dilated columnar epithelial cells (original magnification, ×400). Middle, a typical example of nasopharyngeal carcinoma that is negative for p16. Right, an example of nasopharyngeal carcinoma exhibits focal immunoreactivity to p16. Both nuclear and cytoplasmic positivity was observed (original magnification, ×400).

B, results of cyclin D1 copy number changes and expression levels in 38 paraffin-embedded primary nasopharyngeal carcinomas. C, results of cyclin D1 and p16 expression in 38 primary nasopharyngeal carcinomas.
References


Cyclin D1 Amplification in Nasopharyngeal Carcinoma
# Array-Based Comparative Genomic Hybridization Analysis Identified Cyclin D1 as a Target Oncogene at 11q13.3 in Nasopharyngeal Carcinoma

Angela Bik-Yu Hui, Yvonne Yan-Yan Or, Hirokuni Takano, et al.


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