A Functional Variant in the Transcriptional Regulatory Region of Gene LOC344967 Cosegregates with Disease Phenotype in Familial Nasopharyngeal Carcinoma

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Abstract
Nasopharyngeal carcinoma is a common malignancy in Southeast Asian countries, and genetic background is a well-known component of the complexity underlying its tumorigenic process. We have mapped a nasopharyngeal carcinoma susceptibility locus to chromosome 4p15.1-q12 in a previous linkage study on nasopharyngeal carcinoma pedigrees. In this study provided in this communication, we screened all the genes in this region, with a focus on exons, promoters, and the exon-intron boundary to identify nasopharyngeal carcinoma–associated mutations or functional variants. Importantly, we found a novel gene (LOC344967) with a single nucleotide polymorphism –32G/A in the promoter region. This gene is a member of the acyl CoA thioesterase family that plays an important role in fatty acid metabolism and is involved in the progression of various types of tumors. The –32A variant was found cosegregated with the disease phenotype in the nasopharyngeal carcinoma pedigrees that we previously used for the linkage study. Moreover, this –32A variant creates an activator protein (AP-1)–binding site in the transcriptional regulatory region of LOC344967, which significantly enhanced the binding of AP-1 to the promoter region and the transcription activity of the promoter in vivo. Furthermore, the expression of LOC344967 was significantly up-regulated at both mRNA and protein levels in nasopharyngeal carcinoma cells sharing the –32G/A genotype compared with nasopharyngeal carcinoma cells with the –32G/G genotype. Collectively, these results provide evidence that the –32A variant is a functional sequence change and may be related to nasopharyngeal carcinoma susceptibility in the families studied.

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Introduction
Nasopharyngeal carcinoma is a human malignancy derived from epithelia cells and is rare in most parts of the world but is common in Southern China, Southeast Asian, and in some North African countries (1). Nasopharyngeal carcinoma represents a superb model of gene-environment-virus interaction in the pathogenesis of cancer. Consumption of salted fish has been revealed as a risk factor for nasopharyngeal carcinoma. EBV has been regarded as related to and, perhaps, a probable cause of nasopharyngeal carcinoma. A vast amount of evidence strongly suggests that genetic factors provide a background susceptibility on which EBV and environmental factors act on and eventually result in nasopharyngeal carcinoma development (2–5).

However, how and what genes contribute to nasopharyngeal carcinoma–related genetic susceptibility still remains to be fully clarified. In 1975, Simons et al. first reported the association between the HLA locus and nasopharyngeal carcinoma risk (6), and a number of subsequent studies have confirmed this finding (7–10). Moreover, Lu et al. carried out an affected sib pair linkage analysis, and their results suggested a linkage between the HLA region and the nasopharyngeal carcinoma phenotype (11). Very recently, Lu et al. further localized the susceptibility locus to a 132-kb segment containing the HLA-A locus, but the exact gene has yet to be identified (12–14). Besides the HLA locus or hyplotypes, a susceptibility locus for familial nasopharyngeal carcinoma has been mapped to chromosome 3p21 (15), and polymorphism of the genes of T-cell receptors (16), glutathione S-transferase MI (17), cytochrome P450 2EI (18), polymeric immunoglobulin receptor (19), stress protein HSP70-2 (20), DNA repair enzymes XRCC1 and hOGG1 (21), and certain other genes (22–25) have also been reported to be associated with risk of nasopharyngeal carcinoma. A most likely scenario would be that a number of different genes are involved in different signal pathways or cellular function activities, thus, together would contribute to define a genetic specificity for nasopharyngeal carcinoma; such is in support for a multifactorial mode of inheritance (26).

Familial clustering of nasopharyngeal carcinoma has been well documented (27, 28), and in our previous study on 2,252 nasopharyngeal carcinoma probands, we found that nasopharyngeal carcinoma tended to aggregate in Cantonese families more than in other dialect-speaking populations in Guangdong Province of Southern China (29). By genome-wide linkage analysis with microsatellite markers on 32 Cantonese nasopharyngeal carcinoma pedigrees, we mapped the nasopharyngeal carcinoma susceptibility locus to chromosome 4p15.1-q12 (30). Subsequently, by adding more microsatellite and single nucleotide polymorphism (SNP) markers, we further narrowed down the susceptibility region from 14.21 to 8.29 cM at 4p11-p14 (31). As a result of the progress of the Human Genome Project, we were able to acquire the genomic information of this region; the list of the genes in the region has been published very recently in Nature (32). In an effort to identify the nasopharyngeal carcinoma–associated mutations or functional

Note: B-C. Jiang, H-D. Qin, and M-S. Zeng contributed equally to this work.

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variants, we began to screen all the genes in this region with a focus on exons, promoter, and the exon-intron boundary. In this communication, we report our finding of a novel gene, LOC344967, with a SNP –32A/G in the promoter region cosegregated with the disease haplotype in three largest nasopharyngeal carcinoma pedigrees we previously used for the genome-wide linkage study. Moreover, this SNP –32A results in an activator protein (AP-1)–binding site that significantly enhances the binding of AP-1 to the promoter region in vitro and increases transcription activity in vivo. Thus, our results identify a functional sequence variant that may contribute substantially to susceptibility to familial nasopharyngeal carcinoma.

Materials and Methods

Nasopharyngeal carcinoma pedigrees. The 32 nasopharyngeal carcinoma families were recruited from the Pearl River area in Guangdong Province, China, and they all speak the Cantonese dialect. These families were used for our previous genome-wide linkage study. Detailed descriptions of their clinicopathologic characteristics were reported by Feng et al. (30).

SNP analyses. Screening for SNP was first done on six individuals selected from the two largest pedigrees, 31 and 34; such contributed to the linkage results in a previous study (30). Subsequently, the candidate SNPs were expanded to all the 32 pedigrees for further confirmation. The cases from pedigree 31 include 31-35 and 31-44; 31-15 is a normal control that does not share the disease haplotype. The cases from pedigree 34 include 34-5 and 34-9; the control is 34-11 (30). We focused on the interval spanning from D4S2950 to D4S2916 (37-55 Mb), 34-5 and 34-9; the control is 34-11 (30). We focused on the interval spanning from D4S2950 to D4S2916 (37-55 Mb), 34-5 and 34-9; the control is 34-11 (30).

Preparation of nuclear extracts.

Plasmid constructions.

DNA PCR and mRNA reverse transcription-PCR.

Electrophoretic mobility shift assay. Single-stranded, complementary oligonucleotide sequences corresponding to the 21-bp fragments with the two different alleles (G and A) were synthesized (Shanghai Bioasia Biotechnology Co., Ltd., Shanghai, China) and annealed to form double-stranded probes. Unlabeled consensus AP-1 motif double-stranded oligonucleotides (1.75 pmol/μL) were purchased from Promega. AP-1 antibodies specific for individual components of AP-1 (c-fos and c-jun) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Radioactive probes were generated by labeling a single-stranded oligonucleotide with a T4 polynucleotide kinase and [γ-32P]ATP (5,000 Ci/mmol at 10 mCi/mL, from Beijing FuRui Co., Beijing, China) and subsequently annealing the complementary oligonucleotide and purification through a Sephadex G25 Quickspin column (Roche Diagnostics Ltd., Indianapolis, IN). Binding reactions were done for 10 minutes at room temperature using 5 to 10 μg of HEK293 cell nuclear extract and 30,000 counts/min radiolabeled probe in a binding buffer containing 10 mmol/L Tris-HCl (pH 7.5), 50 mmol/L NaCl, 1 mmol/L MgCl2, 0.5 mmol/L EDTA, 4% glycerol, 0.5 mmol/L DTT, and 0.1 mg poly(deoxyinosinic-deoxyctydilic acid). In competition experiments, a cold competitor (50× and 200× molar excess) was incubated with the nuclear extract for 10 minutes before the addition of the labeled probe. In supershift experiments, 1.0 μg antibody was incubated with the nuclear extract for 3 hours at 4°C after probe addition. Protein/DNA complexes were separated in 6% nondenaturing polyacrylamide gels. The gels were dried and exposed to X-ray films at –80°C overnight. The sequences of the oligonucleotide probes are as follows: AP-1-like oligo (alleles A), 5′-GCTAAT-GTCTGCAGCGCAATGTGCGT-3′; oligonucleotide probe (alleles G), 5′-GCTAATGT CAGCGCAATGTGCGT-3′; and the expected product size is 300 bp (35).

Transient transfection and luciferase assay. For the luciferase assay, HEK293 cells were seeded in six-well microplates 1 day before transfection and grown to 70% to 80% confluence. Either one of the LOC344967 promoter-luciferase constructs (1.6 μg) was cotransfected with 0.4 μg of the pEFRL Renilla luciferase as an internal control for transfection efficiency by 2.0 μL LipofectAMINE 2000. To assess background level of luciferase activity, pGL3-Basic vector without enhancer and/or promoter regions of the LOC344967 gene was cotransfected with the pEFRL Renilla luciferase vector. Cells were harvested 24 hours after transfection, and luciferase activities were determined by the Dual-Luciferase Reporter Assay System (Promega) in a Monolight 3010 luminometer. Firefly luciferase activities were normalized by Renilla luciferase activities. All transfection experiments were done a minimum of three times.

DNA PCR and mirRNA reverse transcription-PCR. DNA and total RNA were isolated from NP69, CNE1, and CNE2 cells. The first-strand cDNA synthesis was done with the SuperScript First-Strand Synthesis System (Invitrogen, San Diego, CA). DNA was amplified with primer pairs (5′-TCATCTCACTTAGGAGGATT-3′ and 5′-GGCTCTATTTGCTGAGGCC-3′) to identify the allele at –32 bp. Reverse transcription-PCR (RT-PCR) was used to analyze LOC344967 gene transcription level with the primer pairs (5′-CTCTGGACATTGGAAGGGATT-3′ and 5′-GGCTCTATTTGCTGAGGCC-3′). The 364-bp RT-PCR product was sequenced for confirmation.

Electrophoretic mobility shift assay. Single-stranded, complementary oligonucleotide sequences corresponding to the 21-bp fragments with the two different alleles (G and A) were synthesized (Shanghai Bioasia Biotechnology Co., Ltd., Shanghai, China) and annealed to form double-stranded probes. Unlabeled consensus AP-1 motif double-stranded oligonucleotides (1.75 pmol/μL) were purchased from Promega. AP-1 antibodies specific for individual components of AP-1 (c-fos and c-jun) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Radioactive probes were generated by labeling a single-stranded oligonucleotide with a T4 polynucleotide kinase and [γ-32P]ATP (5,000 Ci/mmol at 10 mCi/mL, from Beijing FuRui Co., Beijing, China) and subsequently annealing the complementary oligonucleotide and purification through a Sephadex G25 Quickspin column (Roche Diagnostics Ltd., Indianapolis, IN). Binding reactions were done for 10 minutes at room temperature using 5 to 10 μg of HEK293 cell nuclear extract and 30,000 counts/min radiolabeled probe in a binding buffer containing 10 mmol/L Tris-HCl (pH 7.5), 50 mmol/L NaCl, 1 mmol/L MgCl2, 0.5 mmol/L EDTA, 4% glycerol, 0.5 mmol/L DTT, and 0.1 mg poly(deoxyinosinic-deoxyctydilic acid). In competition experiments, a cold competitor (50× and 200× molar excess) was incubated with the nuclear extract for 10 minutes before the addition of the labeled probe. In supershift experiments, 1.0 μg antibody was incubated with the nuclear extract for 3 hours at 4°C after probe addition. Protein/DNA complexes were separated in 6% nondenaturing polyacrylamide gels. The gels were dried and exposed to X-ray films at –80°C overnight. The sequences of the oligonucleotide probes are as follows: AP-1-like oligo (alleles A), 5′-GCTAAT-GTCTGCAGCGCAATGTGCGT-3′; oligonucleotide probe (alleles G), 5′-GCTAATGT CAGCGCAATGTGCGT-3′; and the expected product size is 300 bp (35).

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Quantification of LOC344967 expression using real-time RT-PCR in cell lines with −32G/A or G/G genotypes. Quantitative real-time RT-PCR analysis was done using the Rotor-Gene3000 and SYBRgreen systems (Corbett Research, Sydney, Australia) in accordance with manufacturer’s instructions. Data were normalized by the level of β-actin expression in each of the individual samples. We designed primers using the primer design software Primer Express 2.0 (PE Applied Biosystems). To ensure that the primer amplified the desired cDNA segment, the forward and reverse primers were located on different exons and were checked in the BLAST program in NCBI and Celera database. There was only one PCR product from the pair of primers as seen by electrophoresis, and the product was sequenced to confirm that the correct gene was amplified. Primers for LOC344967 were used as the following: 5'-CGCGGAGGGTGTTCA-3' (sense) and 5'-GATGGCCGTCTGGCTTCT-3' (antisense). Primers for β-actin were used as the following: 5'-CTGTAGCCCAACAGTGCC-3' (sense) and 5'-ATACCTGCTTTGCTGATCC-3' (antisense).

Generation of antigen peptides and antibodies and Western blotting detection. According to mRNA sequence and amino acid sequence of AK125299 (gi: 34531438; NEDO human cDNA sequencing project), the antigen peptide (amino acids 102-118, N-CYPFQEEQGKRTYK) was designed and synthesized by Proteintech Group. Inc. (Chicago, IL). Cysteine was added for keyhole limpet hemocyanin (KLH) conjugation purpose in the peptide. Polyclonal antiserum (S107) to hLOC344967 was obtained after immunization of rabbits with the KLH-coupled internal peptide of hLOC344967 (Proteintech Group). Protein concentrations of cell lysate from the two largest nasopharyngeal carcinoma pedigrees 31 and 34, individuals (four cases and two controls), who were chosen from the susceptibility locus we previously mapped to chromosome 3p21/21p. For the potential nasopharyngeal carcinoma susceptibility haplotype in pedigrees 31, 34, and 62 were found to carry the SNP −32A in G6PC4. These families are the three largest pedigrees in terms of case numbers; in addition, they were the main contributors to the total logarithm of odds (LOD for linkage, and 3.0 was usually considered as a threshold for significance) score of 3.67 in our previous linkage study. LOD scores of pedigrees 31, 34, and 62 were 1.646271, 1.618488, and 0.700149, respectively, and the probabilities of linkage were 0.9659, 0.9637, and 0.7622, respectively (30). These results indicate that −32A is genetically linked to the nasopharyngeal carcinoma phenotype in some of the high-risk nasopharyngeal carcinoma pedigrees. In the remaining 29 pedigrees, about one third of the cases share the −32A variant, and because of the small size of the pedigrees and the ambiguity in linkage, we did not expect all the cases to share the variant as in the three largest pedigrees.

Transcription factor AP-1 binding to the −21/−41 bp region with allele A of the LOC344967 promoter. We then investigated the potential role of the SNP −32A by examining the promoter region with the MatInspector V2.2 program of the TRANSFAC database. We found that the variation −32A in the primary genomic sequence resulted in a potential binding site for transcriptional factor AP-1 in the region of −26/−36 bp of LOC344967 5'-untranslated region (Fig. 3A). We synthesized two oligonucleotides containing the −21 to −41 region with the −32A or −32G allele and carried out a gel shift assay. As shown in Fig. 3B, the 32P-labeled oligonucleotide (allele A) bound specifically to nuclear extracts from HEK293 cells and formed a shifted complex at the same position as the AP-1 consensus oligonucleotide. But the oligonucleotide with allele G did not form such a complex (Fig. 3B, lane 5). This was confirmed by supershift with antibodies to AP-1 and a complete inhibition by 200-fold unlabeled consensus AP-1 oligonucleotide and the allele A-containing oligonucleotide (Fig. 3B).

To test whether the binding also occur in vivo, we carried out a chromatin immunoprecipitation assay on one nasopharyngeal carcinoma cell line CNE2 with −32G/G genotype and one immortalized nonmalignant nasopharyngeal epithelial cell line NP69 with −32G/A genotype. As shown in Fig. 4, a clear PCR amplification product of the LOC344967 promoter was observed in chromatin immunoprecipitation carried out with anti-AP-1 antibody only in NP69 cells but not in CNE2 cells (Fig. 4, bottom, lanes 5 and 9). For the positive control, MSH2 promoter (35), a clear band was observed in both CNE2 and NP69 cells (Fig. 4, top, lanes 5 and 9) but not for the negative controls, rabbit serum, or PBS (Fig. 4, top and bottom, lanes 3, 4, 7, and 8). Collectively, these data suggest that there was an AP-1-binding motif at the −21/−41 bp region because of the substitution −32G/A, and transcription factor AP-1 was able to specifically bind to this region in vitro and in vivo.

−32A variant located in the promoter region of LOC344967 generated one functional cis-regulatory element and was associated with greater promoter activity. To further determine
the effects of the −32A variant on AP-1-induced LOC344967 transcriptional activation, transient transfection assays with the luciferase reporters driven by the LOC344967 promoter segment containing either allele A or G were done in HEK293 cells. We cloned both the −1,008-bp region containing −32A or G of the LOC344967 promoter into the pGL3 vector upstream of the luciferase reporter. Those constructs were transfected into the HEK293 cells, and luciferase activity was measured 24 hours after transfection. As we expected, the luciferase activity of the construct with allele A was 1.83 times greater than that with allele G (P < 0.0001; Fig. 5). These results suggest that the −32A variant is functionally significant.

LOC344967 is widely expressed in multiple tissues, and its mRNA and protein expression levels were associated with the −32A variant. To investigate the tissue distribution of LOC344967, we detected LOC344967 mRNA expression in multiple tissues and in the nasopharyngeal carcinoma cell lines by RT-PCR analysis. LOC344967 transcript was detected in all of the tissues, including liver, lung, kidney, brain, and intestine, and in several
nasopharyngeal carcinoma cell lines (data not shown). This wide expression pattern may indicate an important role for this gene. Next, we sought to determine if there is a relationship between the −32 variant and the mRNA expression levels. For this purpose, we checked and found two nasopharyngeal carcinoma cell lines CNE1 and CNE2 carrying the −32G/G and two cell lines NP69 and C666 carrying the −32G/A genotypes (Fig. 6A). These cell lines are very popularly used in the field of nasopharyngeal carcinoma research, and three of them are derived from nasopharyngeal carcinoma cells, and only NP69 is a SV40 T antigen –immortalized nasopharyngeal epithelial cell line. Quantitative real-time RT-PCR analysis was used to detect the expression levels of LOC344967. We found a significantly greater expression level in the −32 G/A heterozygous cells NP69 and C666 than in the homozygous −32G/G cells CNE1 and CNE2 (Fig. 6B). Furthermore, we designed a peptide to make polyclonal antibodies, based on analysis of the putative amino acid sequence of LOC344967. The molecular weight of the encoded protein of LOC344967 is ~28 kDa (252 amino acids); a protein band of similar size was detected by Western blotting with the antibody. As shown in Fig. 6C, the protein levels in NP69 and C666 cells were notably higher than in CNE1 and CNE2 cells.

Discussion

Nasopharyngeal carcinoma is a complex disease with a proven genetic component. This disease is regarded by many to be an excellent and has been regarded as a good model for studying gene-environment-virus mechanistic interactions. However, the precise genetic component in this disease has remained largely elusive. In the elucidation of this genetic component, we have considered two alternative hypotheses: (a) only one copy of the susceptibility allele has survived in the high-risk population, with long-conserved haplotypes being observed, such has been shown in previous studies of other complex diseases (37–39). (b) The common disease/common variant hypothesis, suggesting a founder effect that is common to the high-risk population and that complex traits have underlying genetics variants with high frequency. Such mutations may have occurred some 100,000 years ago, undergoing little or no selection during population expansion (40). Whether the rare variants or the common variants account for the genetic complexity has been discussed extensively. The variant −32A, in gene LOC344967, as identified in this study, matched well with the nasopharyngeal carcinoma disease phenotype (Figs. 1 and 2). This suggests that the variant might be a high risk factor in high-risk familial nasopharyngeal carcinoma. We have previously carried out a complex segregation analysis based on 1,903 Cantonese pedigrees, and our results provided evidence for a multifactorial mode of inheritance (26). We may assume that −32A was one of such risk factors, and this factor, together with sequence variants

Figure 3. Transcription factor binding to the putative AP1-like motif in the −41/−21 region of the LOC344967 promoter. A, schematic illustration of the −54/3 region of the LOC344967 promoter indicating positions of the putative AP1 transcription factor binding motif (underlined). The sequences of wild-type (G) and mutant (A) (in red) oligonucleotide probes used for gel shift analysis is indicated by the brackets. Nucleotide numbering is according to AK125299 sequences (gi: 34531348. NEDO Human cDNA Sequencing Project). B, Gel shift, supershift and competition assays with HEK293 cell nuclear extract and oligonucleotides with different allelic variants of the LOC344967 promoter. Binding was present with the AP1-like oligonucleotide containing allele A (lane 4, 7 and 9) and AP1 consensus oligonucleotide (lane 3), but was not observed with oligonucleotide containing allele G (lane 5) when HEK293 cell extract was present. Binding to oligonucleotide with allele A resulted in a complex that was completely competed (cold oligos) by 300-fold molar excess of unlabeled AP1-like oligonucleotide containing allele A (lane 8) and AP1 consensus oligonucleotide (lane 6), but was not competed by the same amount of unlabeled oligonucleotide containing allele G (lane 9). AP1-like oligonucleotide containing allele A supershift was present with antibodies against c-Jun (lane 11) and c-Fos (lane 12). AP1 consensus oligonucleotide supershift was present with antibodies against c-Jun (lane 10) and c-Fos (lane 12).

Figure 4. ChIP analysis of AP1 binding to the putative AP1-like motif of the LOC344967 promoter. CNE2 and NP69 cells were cultured and chromatin lysates of these two cell lines were prepared as described under "Materials and Methods." Equal aliquots of chromatin lysates were subjected to immunoprecipitation with antibodies against AP1 (anti-c-Jun/anti-c-Fos mixture) (lane 5 and 9), rabbit serum control (lane 4 and 8) or blank (lane 3 and 7) with PBS control. The DNA associated with immunoprecipitates was isolated and used as templates to PCR-amplify the positive control gene MSH2 promoter region containing a known functional AP1 binding site (upper panel) or the LOC344967 promoter containing the putative AP1-like motif (lower panel). The PCR products were resolved by agarose gel electrophoresis and stained with ethidium bromide.
in other genes, infection of certain subtypes of EBV, and exposure to environmental carcinogens, could eventually result in nasopharyngeal carcinoma development (3). Certainly, at this stage, we could not rule out the possibility of certain rare mutations with a much higher risk existing in the susceptibility region. One rare variant CAT26CAA (His26Gln) was identified in \textit{COX7B2} gene in pedigree 31 but not in other pedigrees nor sporadic nasopharyngeal carcinoma, and its real relationship with nasopharyngeal carcinoma needs to be further determined (36).

In addition to the genetic evidence, supporting a role of the variant \textit{−32A} of \textit{LOC344967} in relationship with familial nasopharyngeal carcinoma, we have also provided functional evidence. Our \textit{in vitro} gel shift, supershift, and competition assay and \textit{in vivo} chromatin immunoprecipitation assay have clearly showed that the AP-1-binding site was formed from the variant compared with the \textit{−32G} sequence (Figs. 3 and 4). Furthermore, our construct containing the \textit{−1,008-bp} promoter region with the \textit{−32A} allele and a luciferase reporter produced significantly greater luciferase activity than the construct with the \textit{−32G} allele (Fig. 5). These results indicate an enhanced transcription of \textit{LOC344967} with the \textit{−32A} allele through the newly formed AP-1-binding site. The rationale to link such a mechanism to nasopharyngeal carcinoma development may be explained, at least in part, by the oncogenic potential of the EBV-encoded LMP1 protein. LMP1 has been reported to be an important protein, tightly linked to apoptosis prevention and malignant transformation, through activating transcription factors nuclear factor-\kappaB (NF-\kappaB) and AP-1 (41–43). In addition, a mutant at amino acids 204, 206, 208, and 384 has been reported to significantly inhibit LMP1-stimulated NF-\kappaB and AP-1 transcriptional activity (41). Furthermore, a SNP in matrix metalloproteinase-1 promoter creates an Ets-binding site for LMP1 resulting in enhanced susceptibility to nasopharyngeal carcinoma (44). On the other hand, the most abundant polyadenylated viral transcripts of EBV, the so-called complementary-strand transcripts,

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contain a functional AP-1 regulatory element in its promoter region (45). Thus, it seems that AP-1 plays a central role and is substantially involved in EBV-induced cell transformation. An important question is how an up-regulated LOC344967 might contribute to nasopharyngeal carcinoma development. By sequence alignment, LOC344967 shares similarity to a cytosolic acyl-CoA thioester hydrolase (EC 3.1.2.2, located in chromosome 1p36.31-p36.11; refs. 46, 47), which belongs to a family containing a wide variety of enzymes, principally the thioesterases. This family includes various cytosolic long-chain acyl-CoA thioester hydrolases. Long-chain acyl-CoA hydrolases hydrolyze palmitoyl-CoA to CoA and palmitate and catalyze the hydrolysis of other long-chain fatty acyl-CoA thioesters. These enzymes can be present in a membrane-bound form in subcellular organelles and in a soluble form inside mitochondria and cytosol (46). An important function of these enzymes is determining the chain length of the synthesized fatty acids. Previous studies have established a link between cancer development and fatty acid synthesis (48). For example, fatty acid synthase (FAS) has been reported to be up-regulated in prostate cancer cells compared with normal prostate epithelial cells and has been implicated in the progression of various types of tumors (49–52). Orlistat, an inhibitor of the thioesterase activity of FAS, can block cellular fatty acid synthesis; this results in an inhibition of prostate tumor cell proliferation and an induction of apoptosis in prostate tumor cells (53). CoA thioesterase activity has also been reported to be induced by hypolipidemic peroxisome–proliferating agents leading to an activation of retinoid X receptors and an initiation of transcription of downstream molecules (46). Furthermore, EBV-encoded BZLF1 can physically and functionally interact with the retinoic acid receptors (54).

With regard to LOC344967, although its biological function remains to be determined, we have successfully detected its expression in a variety of normal human tissues, nasopharyngeal carcinoma biopsy specimens, and nasopharyngeal carcinoma cell lines by the RT-PCR method (data not shown). The widely expressed pattern of LOC344967 suggests a potentially important function of this gene. Considering the ubiquitous existence of the AP-1 transcription factor, we suggest that the ∼32A variant could result in changes in the expression level of LOC344967. To test this hypothesis, we selected four cell lines with two sharing the ∼32G/G and two sharing the ∼32G/A genotypes. Each cell line was derived from nasopharyngeal epithelial cells: three are malignant epithelial cell lines and one is immortalized epithelial cell line. LOC344967 expression levels were significantly up-regulated at both the mRNA and protein levels in the two cell lines containing ∼32G/A genotype. It is conceivable, therefore, that the up-regulation of LOC344967 could result in higher enzyme activity in the epithelial cells, substantially altering fatty acid metabolism and consequently promoting neoplastic cell transformation. Undoubtedly, future studies will more firmly establish the relationship between fatty acid metabolism and epithelial cell–derived cancer development, such studies will shed light on the extremely complicated mechanism of cancer development, which, most probably, will involve multiple genes related to various cellular activities, including, potentially, fatty acid metabolism.

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