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Review

Tumor suppressor genes on frequently deleted chromosome 3p in nasopharyngeal carcinoma

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Abstract

Nasopharyngeal carcinoma (NPC) is among the most common malignancies in southern China. Deletion of genomic DNA, which occurs during the complex pathogenesis process for NPC, represents a pivotal mechanism in the inactivation of tumor suppressor genes (TSGs). In many circumstances, loss of TSGs can be detected as diagnostic and prognostic markers in cancer. The short arm of chromosome 3 (3p) is a frequently deleted chromosomal region in NPC, with 3p21.1–21.2 and 3p25.2–26.1 being the most frequently deleted minimal regions. In recent years, our research group and others have focused on the identification and characterization of novel target TSGs at 3p, such as *RASSF1A*, *BLU*, *RBMS3*, and *CHL1*, in the development and progression of NPC. In this review, we summarize recent findings of TSGs at 3p and discuss some of these genes in detail. A better understanding of TSGs at 3p will significantly improve our understanding of NPC pathogenesis, diagnosis, and treatment.

Key words Tumor suppressor gene, deletion of 3p, nasopharyngeal carcinoma

Nasopharyngeal carcinoma (NPC) is a common head and neck malignancy in Southeast Asia, especially in southern China where it reaches a peak incidence of 20 to 30 cases per 100 000 people [1,2], but is rare in Europe and North America [3]. Several etiologic factors have been associated with NPC, including early infection with Epstein-Barr virus (EBV), genetic predisposition, chemical carcinogens in some traditional diets (particularly salted fish) [4], and exposure to noxious inhalants [5]. According to the World Health Organization (WHO) classification, NPC is classified as keratinizing or nonkeratinizing. The latter includes poorly differentiated (WHO type II) and undifferentiated (WHO type III) nonkeratinizing carcinoma. Poorly differentiated non-keratinizing carcinoma is more common in southern China, accounting for more than 97% of NPC cases [6]. This NPC epidemic also shows familial aggregation; the risk of NPC is more than 9 times as high in first-degree relatives of the patient compared to first-degree relatives of the spouse [7]. The majority (75% to 90%) of newly diagnosed NPC patients have loco-regionally advanced disease, commonly with cervical nodal metastases [8], indicating that most NPC patients are not promptly diagnosed.

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Recurrent Genetic Alterations in NPC

Although several factors including EBV infection, environmental risks, and genetic susceptibility have been associated with NPC [9-12], the molecular mechanisms underlying the development of NPC remain unclear. Cytogenetic studies have shown that multiple chromosomal abnormalities were frequently detected in individual cases, suggesting that genomic instability might play an important role in NPC pathogenesis [13,14]. During last two decades, the importance of chromosomal aberrations in the pathogenesis of cancer has been widely elucidated. Like most cancers, NPC is a heterogeneous disease with complex genetic changes. Therefore, identifying and characterizing recurrent genomic alterations is a useful strategy to explore genes involved in the development and progression of NPC. In

Table 1, we summarized the recurrent genomic changes in NPC detected by comparative genomic hybridization (CGH) studies. These genomic alterations include gains of 1q, 2q, 3q, 4q, 5q, 6q, 7q, 8, 11q, 12, and 17q and losses of 1p, 3p, 9p, 9q, 11q, 13q, 14q, and 16q [15-21].

Among these chromosomal abnormalities, 3p is the most frequently deleted region (Figure 1), implying that 3p may harbor one or more TSGs. In addition, loss of 3p has been detected as an early and critical molecular event in the pathogenesis of NPC [22-24], and has been

Table 1. Summary of comparative genomic hybridization (CGH) results in nasopharyngeal carcinoma (NPC)

Chromosomal alteration	Prevalence of chromosomal alteration reported in literature						
	Ref. [15] (*n*=51)	Ref. [16] (*n*=20)	Ref. [17] (*n*=47)	Ref. [18] (*n*=30)	Ref. [19] (*n*=10)	Ref. [20] (*n*=17)	Ref. [21] (*n*=10)
Gains							
1q	47%	40%	32%	20%	60%	35%	80%
2q	–	20%	–	23%	50%	29%	20%
3q	–	30%	34%	20%	70%	29%	30%
4q	20%	17%	–	–	–	59%	–
6q	–	25%	–	13%	50%	–	–
7q	–	20%	–	13%	40%	–	10%
8p	–	30%	–	10%	–	–	–
8q	–	30%	–	27%	60%	29%	10%
11q	41%	–	–	–	80%	35%	20%
12p	59%	60%	–	36%	–	–	40%
12q	35%	60%	51%	33%	80%	41%	10%
17q	47%	10%	–	–	60%	–	–
Losses							
1p	–	30%	43%	–	–	82%	20%
3p	53%	75%	43%	20%	50%	53%	40%
9p	41%	25%	–	–	50%	–	20%
9q	–	40%	–	–	–	29%	10%
11q	29%	45%	36%	23%	80%	47%	40%
13q	41%	35%	–	–	–	35%	40%
14q	35%	65%	21%	13%	50%	47%	40%
16q	–	50%	55%	16%	–	29%	10%

"–" refers to undetectable.

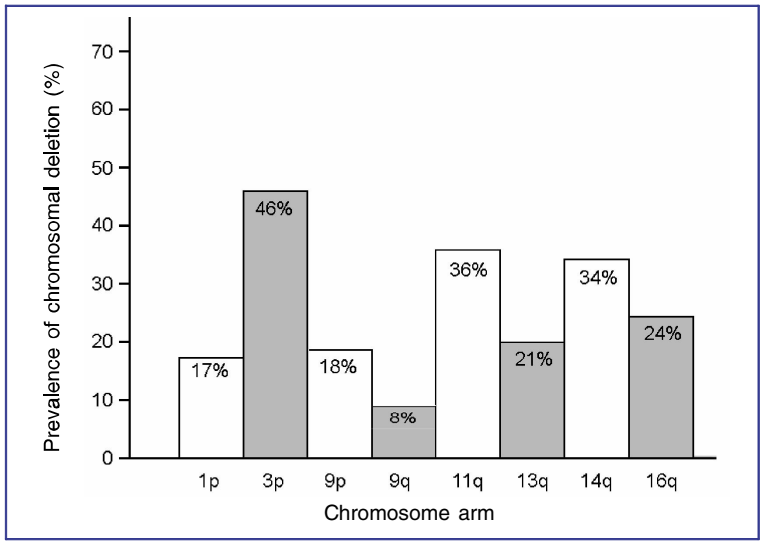

Figure 1. **Summary of genomic deletions in 185 nasopharyngeal carcinoma (NPC) cases.** Deletion of 3p is the most frequently detected chromosomal alteration.

associated with a significantly higher risk of death from recurrent tumor compared with patients without 3p loss [24].

Frequently Deleted Minimal Regions at 3p in Human Cancers

By using CGH, scientists have demonstrated that copy number loss of chromosome 3p is a frequent alteration in NPC and has been suggested as an early genomic alteration during NPC progression. Using microsatellite markers, several frequently deleted minimal regions at 3p were identified in NPC, including 3p25.2–26.1 (51.3%), 3p21.1–21.2 (51.3%), 3p14.3–21.1 (48.7%), 3p21.3 (47.4%), and 3p26.2–26.3 (45%)[25,26]. In lung cancer, several frequently deleted regions, including 3p26.3, 3p25.3, 3p24.1, 3p23, and 3p21.1, have been identified by SNP-MassArray[27]. Another loss of heterozygosity (LOH) study in lung cancer showed that frequently deleted regions at 3p include 3p24–26, 3p21.3, 3p21.1–21.2, 3p14.2, and 3p12–13 [28]. In esophageal squamous cell carcinoma (ESCC), deletion of 3p is also a frequent allelic imbalance detected by CGH[29-31], LOH[32], and genome-wide genotyping[33]. Using SNP-MassArray, several commonly deleted regions in ESCC have been identified, including 3p26.3, 3p22, 3p21.3, and 3p14.2 [33]. Furthermore, deletions of 3p22 and 3p14.2 have been related with advanced tumor stage, and deletion of 3p22 was found to associate with tumor metastasis in ESCC [33]. In pancreatic endocrine tumor, deletion of 3p, especially at the 3p21.1– 21.3 and 3p 25.2–26.1 regions, is a frequently detected genomic alteration in advanced stage disease [34]. These results suggest that frequently deleted regions may harbor one or more TSGs that play important roles in the pathogenesis of various solid malignancies, including NPC.

Identification of NPC-related TSGs at 3p

In human solid tumors, copy number alterations are believed to contribute to tumorigenesis by affecting the functions of cancer-related genes in altered chromosomal regions [35]. In NPC, isolating and identifying putative cancer-related TSGs will help us to understand its pathogenesis. During the last decade, several candidate TSGs at 3p21.3 have been identified and characterized, including *RASSF1A* [Ras association (RalGDS/AF-6) domain family member 1], *GNAT1* [guanine nucleotide binding protein (G protein), alpha transducing activity polypeptide 1], *BLU* (zinc finger, MYND-type containing 10), and *LARS2* (leucyl-tRNA synthetase 2, mitochondrial), which are inactivated by promoter hypermethylation and/or LOH in NPC [36-40]. Several other TSGs at 3p have been identified, including *NAG7* (long intergenic non-protein coding RNA 312) at 3p25[41], *LTF* (lactotransferrin) at 3p21[42,43], *FBLN2* (fibulin 2), *TMEM45A* (transmembrane protein 45A), *ZIC4* (Zic family member 4), *GPR149* (G protein-coupled receptor 149), and *ETV5* (ets variant 5) [44]. Recently, in our laboratory, candidate TSGs *CACNA2D3* (calcium channel, voltage-dependent, alpha 2/delta subunit 3) at 3p21.1, *RBMS3* (RNA-binding motif, single stranded interacting protein 3) at 3p33, and *CHL1* [cell adhesion molecule with homology to *L1CAM* (close homolog of L1)] at 3p26 have been identified (unpublished data) (Table 2).

Complex Progression of NPC Carcinogenesis

The development and progression of NPC is a complex process. EBV is a ubiquitous herpesvirus that preferentially infects oropharyngeal epithelial cells and B-lymphocytes in humans. The virus is present in all NPC tissues, which usually includes pre-invasive nasopharyngeal lesions [45,46]. Little is known about the underlying reasons why EBV-infected epithelial cells undergo increased proliferation[47]. Recently, EBV was the first virus found to encode microRNAs (miRNAs), short, non-coding RNAs that in most cases negatively regulate gene expression at the post-transcriptional level and guide gene silencing [48]. The most widely accepted hypothesis of NPC development and progression involves a series of sequential steps. The process begins with transformation, which occurs in nasopharyngeal epithelium due to chronic EBV infection or triggering by external stimuli (chemical carcinogens, noxious inhalants, or metabolic diseases). Transformation is followed by a series of hyperplastic and dysplastic stages, and the disease progresses to become more malignant, making metastasis possible. The changes by which a normal cell becomes a cancer cell are thought to be caused by aberrant gene expression critical to cellular processes such as cell cycle control, apoptosis, cell adhesion, migration, and other functions at the genetic, molecular, and cellular levels. These altered cells may undergo clonal expansion and immortalization to develop NPC. Later on, neoplastic cells become more undifferentiated and invasive, and then spread out of the nasopharyngeal epithelium to the brain or other distant locations, which indicates end-stage disease.

Roles of TSGs at 3p during Nasopharyngeal Carcinogenesis

During human multiple complex nasopharyngeal

Table 2. Candidate tumor suppressor genes at 3p in NPC

Gene	Location	Function(s)	Down-regulation	Reference(s)
CACNA2D3	3p21.1	Cell proliferation; cell cycle regulation apoptosis; invasion and metastasis	92.3% (12/13)[a]	Wong et al. (unpublished)
GNAT1	3p21.3	Remains to be revealed	72.7% (24/33)	Dryja et al.[44]; Yi et al.[45]
LTF	3p21.3	Cellular growth, differentiation, protection against cancer development and metastasis	76% (25/33)	Yi et al.[46]; Zhang et al.[47]; Zhou et al.[48]
BLU	3p21.3	Cell proliferation; stress-responsive	83% (24/29)	Liu et al.[51]; Agathanggelou[52]; Qiu et al.[53]; Yau et al.[54];
LARS2	3p21.3	Encodes a class 1 aminoacyl-tRNA synthetase, mitochondrial leucyl-tRNA synthetase	78% (28/36)	Zhou et al.[55]
RASSF1A	3p21.3	Cell proliferation; cell cycle regulation; apoptosis	100% (38/38)	Wang et al.[56]; Hutajulu et al.[57]; Chow et al.[58]
RBMS3	3p24.1	Cell proliferation; cell cycle regulation; apoptosis	86.7% (13/15)	Chen et al. (unpublished)
FBLN2	3p25.1	Cell proliferation; invasion; metastasis and angiogenesis	46.7% (14/30)	Law et al.[39]
CHL1	3p26.1	Cell proliferation; cell cycle regulation; apoptosis; invasion and metastasis	80.0% (12/15)	Chen et al. (unpublished)

[a] The percentage of NPC cases with lower expression of the target gene than their adjacent nontumor tissues (the sum of cases with lower target gene expression divided by the sum of NPC cases examined from all the relevant references).

carcinogenesis, DNA copy loss is a pivotal mechanism by which TSGs become inactivated. In many cases, deleted TSGs are identified as both prognostic markers and tumor therapy targets. In this review, we summarize the function of deleted 3p genes and describe in detail the role of novel candidate TSGs *RBMS3* and *CHL1* identified by our group.

GNAT1

GNAT1 stimulates the coupling of rhodopsin and cGMP-phoshodiesterase during visual impulses and encodes the alpha subunit in rods. Mutations in this gene result in autosomal dominant congenital stationary night blindness [49]. *GNAT1* is expressed stably in all chronic nasopharyngitis tissues, whereas absent or down-regulated in specimens of NPC. LOH of *GNAT1* was correlated to its expression level [50], whereas the functional role of *GNAT1* remains to be revealed.

LTF

LTF, which belongs to the transferrin family, is a major iron-binding protein in milk and body secretions and has an antimicrobial activity, making it an important component of the non-specific immune system. This protein demonstrates a broad spectrum of properties, and two-hit silencing of this gene through genetic and epigenetic changes may be a common and important event in carcinogenesis. LTF inhibited NPC proliferation by inducing cell cycle arrest and modulating the MAPK signaling pathway[50-52]. Studies also highlight the potential for LTF in chemoprevention and suggest that it may become a biologically relevant prognostic marker in prostate cancer[53] and lung cancer[54].

BLU

In 2003, Liu *et al.*[55] found that the *BLU* gene was frequently altered in NPCs; however, there was no evidence of a suppressive effect on NPC cell proliferation. Epigenetic inactivation of *BLU* has been strongly indicated in the pathogenesis of common human cancers and has been observed in the following: lung cancer (39%), breast cancer (42%), kidney cancer (50%), neuroblastoma (86%), and NPC cell lines (80%)[56]. *BLU* was also found to be activated by environmental stresses such as heat shock, which was regulated by E2F [39]. Furthermore, *in vivo* studies provided the first significant evidence to demonstrate that *BLU* could functionally suppress tumor formation. Taken together, these findings suggest that *BLU* is likely a candidate TSG involved in NPC[57].

LARS2

LARS2 encodes a class 1 aminoacyl-tRNA synthetase, mitochondrial leucyl-tRNA synthetase. Hypermethylation of *LARS2* was found in 64% of NPC samples but only in 12.5% of non-cancerous

nasopharyngeal biopsies. Inactivation of *LARS2* by both genetic and epigenetic mechanisms may be a common and important event in the carcinogenesis of NPC[58].

RASSF1A

RASSF1A, also named *RASSF1*, encodes a protein similar to the Ras effector proteins. Loss or altered expression of this gene, which is related with hypermethylation of its CpG island promoter region, has been associated with the pathogenesis of a variety of cancers, including NPC[59-62], prostates cancer[63], non-small cell lung cancer[64], and head and neck squamous cells cancer[65], suggesting this gene has a tumor suppressor function. In NPC, *RASSF1A* is one of the five specific methylation markers (*RASSF1A*, *p16*, *WIF1*, *CHFR*, and *RIZ1*) for NPC risk assessment in combination with EBV-based markers[61]. Hypermethylation of *RASSF1A* was related with age at diagnosis and T stage. An *in vitro* study showed that ectopic expression of *RASSF1A* could inhibit tumorigenicity via cell cycle arrest at G_0/G_1 phase and induce apoptosis in a Ras-dependent manner[59].

FBLN2

FBLN2 gene is located at 3p25.1, and the protein it encodes interacts with extracellular matrix (ECM) proteins. Law *et al.*[44] demonstrated that it played a pivotal tumor-suppressive and anti-angiogenic role in NPC. Methylation of *FBLN2* has been observed in breast, colorectal, and lung cancers[66], and deletion of this gene has often been detected in ESCC[67].

RBMS3

RBMS3 gene encodes an RNA-binding protein that belongs to the c-myc gene single-strand binding protein (MSSP) family[68]. These proteins are characterized by the presence of two sets of ribonucleoprotein consensus sequences that contain conserved motifs, RNP1 and RNP2, that were originally described in RNA-binding proteins and are required for DNA binding. MSSP family proteins have many diverse functions and regulate processes such as DNA replication, gene transcription, cell cycle progression, and apoptosis. RBMS3 was isolated by virtue of its binding to an upstream element of the alpha 2 (type I) collagen promoter[69]. It localizes mostly in the cytoplasm, suggesting that it may be involved in a cytoplasmic function, such as controlling RNA metabolism, rather than transcription. Multiple alternatively spliced transcript variants of the *RBMS3* gene that encode various isoforms have been found. However, the relationship between *RBMS3* and NPC has not been revealed.

In our recent study (unpublished), down-regulation of *RBMS3* was detected in all 3 (100%) cell lines and 13 of 15 (86.7%) paired NPC tissues. Functional analysis showed that *RBMS3* suppressed tumorigenicity of NPC cells both *in vitro* and *in vivo*, including inhibiting cell growth, colony formation *in vitro*, and tumor formation in nude mice. At the molecular level, the tumor suppressive mechanism of RBMS3 involved cell cycle arrest at the G_1/S checkpoint induced through up-regulation of Smad4, p53, and p21, down-regulation of cyclin E and CDK2, and subsequent inhibition of Rb phosphorylation at Ser780. Mechanistic investigations also suggested these effects may be mediated by down-regulation of β-catenin and inactivation of its downstream targets, including cyclin D1, c-myc, and MMP7. Furthermore, RBMS3 was found to induce apoptosis in a mitochondrial-dependent manner by activating caspase-9 and PARP. Taken together, our findings reveal *RBMS3* as an important TSG in NPC development.

CHL1

The protein encoded by *CHL1* is a member of the L1 family of neural cell adhesion molecules. It is a neural recognition protein that may be involved in signal transduction pathways. Similarly, the *CHL1* gene is involved in general cognitive activities[70,71] and neurological diseases such as schizophrenia[72]. Deletion of one copy of this gene might be responsible for mental defects in patients with 3p- syndrome. Recently, several cell adhesion molecules, including L1, were shown to be involved in cancer growth and metastasis[73,74]. 3p26 has been reported to harbor a candidate gene for prostate cancer susceptibility in Finnish prostate cancer families; however, no mutations were detected in the coding part of *CHL1*[75]. Nevertheless, these reports suggest that *CHL1* plays a pivotal role in cancer development[33], not only in neuronal activities. In our recent study (unpublished), down-regulation of *CHL1* caused by promoter methylation was observed in both ESCC and NPC. Furthermore, functional study showed that ectopic expression of CHL1 in NPC cells dramatically inhibited their clonogenicity and migration as compared with parental NPC cells without CHL1 expression. These data provide preliminary evidence that *CHL1* has tumor suppressive functions in NPC.

Conclusions and Perspectives

During NPC progression, regional chromosomal loss is a major mechanism by which TSGs are inactivated. Loss of 3p is a frequently detected alteration in NPC and has been identified as an early genomic event

associated with the NPC development [16-18]. Due to the highest frequency of copy number loss at 3p21.1-21.3 and 3p25.2-26.1, much effort has been devoted to identify target genes responsible for 3p deletion. However, the extensive and complex nature of chromosomes makes it difficult to identify the biologically relevant aberrations and functional significance. During this decade, identification and characterization of 3p target genes, such as *RASSF1A*, *GNAT1*, and *BLU*, has been the focus of many researches. Furthermore, efforts remain underway to determine the roles of 3p target genes in NPC progression. Although more and more candidate TSGs lost in 3p have been identified, the precise mechanisms underlying NPC initiation and progression remain unclear. It is also vital to understand coactivation of these TSGs as well as the relationship among EBV, miRNAs, and TSGs in the development of NPC, which has prompted investigation of the microenvironment, immune surveillance and elimination, cancer cell transformation and invasion. With the increasing understanding of viral, genetic, and environmental factors, development of gene-based or miRNA-based therapy may lead to the eradication of NPC and related diseases in the future.

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