Review Article

Tumor suppressor genes in oral cancer

Vinayak Gourish Naik, Prakruti Adhyaru, Ajit Gudigenavar

Department of Oral and Maxillofacial Surgery, Bapuji Dental College and Hospital, Davangere, Karnataka, India

ABSTRACT

The incidence of oral cancer remains high and is associated with many deaths. Several risk factors for the development of oral cancer are now well known, including smoking, drinking and consumption of smokeless tobacco products. Genetic predisposition to oral cancer has been found in certain cases but its components are not yet entirely clear. A number of genomic lesions accompany the transformation into oral cancer and a wealth of related results has appeared in recent literature. Tumor suppressor genes can be grouped into 3 categories like caretaker genes, gatekeeper genes, and landscaper genes; the classification schemes are evolving as medicine advances, learning from fields including molecular biology, genetics, and epigenetics. Tumor suppressor genes in oral cancer have been analyzed a lot in many studies. This review highlights the important areas about tumor suppressor genes in oral cancer and provides an overview on the understanding of these genes.

Key words: Genetic predisposition, oral cancer, tumor suppressor genes

INTRODUCTION

A tumor suppressor gene (TSG), or antioncogene, is a gene that protects a cell from one step on the path to cancer. When this gene mutates to cause a loss or reduction in its function, the cell can progress to cancer, usually in combination with other genetic changes. The loss of these genes may be even more important than proto-oncogene/oncogene activation for the formation of many kinds of human cancer cells.[1] TSGs can be grouped into categories including caretaker genes, gatekeeper genes, and landscaper genes; the classification schemes are evolving as medicine advances, learning from fields including molecular biology, genetics, and epigenetics.[1] TSGs in oral cancer have been analyzed a lot in many studies. The aim of this review is to highlight the importance of TSGs in oral cancer and provide an overview on the understanding of these genes thereby helping potential avenues for further research.

Address for correspondence: Dr. Vinayak Gourish Naik, Department of Oral and Maxillofacial Surgery, Bapuji Dental College and Hospital, P.O. Box #326, Davangere - 577 004, Karnataka, India. E-mail: drvinayakgn@gmail.com

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TUMOR SUPPRESSIVE GENES

The proto-oncogenes encode proteins that promote cell growth and the genes that encode proteins which apply brakes to cell proliferation are called growth regulatory genes, recessive oncogenes, or antioncogenes, but they are most often referred to a TSGs.[2-4] Unlike oncogenes, which can effect a cellular change through mutation of only one of the two gene copies, TSGs are inactivated by point mutations, deletions, and rearrangements in both gene copies in a "two-hit" fashion. Therefore, the critical events for the malignant transformation of oral keratinocytes, the "loss of function" mutations of TSGs, are more difficult to achieve. This may account, in part, for the length of time adult solid tumors such as oral cancers take to form.[3,5]

Many TSGs were initially identified in pediatric tumors that formed early in life because one mutated TSG had already been inherited,[3] e.g., the first and prototypic cancer suppressor gene to be discovered was the retinoblastoma (*Rb*) gene, the discovery of which was accomplished by the study of a rare disease, the Rb (an uncommon childhood tumor).[1]

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However, identification of TSGs occurred a decade behind the isolation of the first oncogenes, because in cancer cells, TSGs are a "negative phenotype" or an event no longer present within the cell. Knudson predicted that the inactivation of both copies of TSGs occurs in a "two-hit" fashion through mathematical models analyzing genetic pedigrees of pediatric tumor patients.[3] Experimental evidence followed in late 1960s, Harris *et al*. carried out fusion of malignant cells with normal cells in culture. They found that malignant phenotype was suppressed in the hybrid cells. This was attributed to the action of TSGs in the normal cells. The loss of this tumor suppressor activity leads to malignancy.[4] These same experiments have been performed with normal and malignant oral keratinocytes to show that TSG loss is necessary for oral carcinogenesis.[3]

Mutations and subsequent inactivation of a TSG cause so-called "loss of function," whereas inactivation of an oncogene gives "gain of function." TSGs are, however, most commonly recessive to the normal allele, meaning that if one allele is mutated its phenotype is not expressed as long as the other allele's nature is of a wild type.[5] Commonly, one allele of a TSG sustains a mutation (heterozygosity) which inactivates the function of its protein and then the second allele is lost via deletion or gene conversion, resulting either in a loss of heterozygosity (LOH) or a reduction to homozygosity at the locus in the cells of the tumor.[6] LOH appears to represent the second genetic inactivation step in the complete loss of a TSG locus.[7] The TSGs *p53* and *Rb* have been the most extensively studied genes.

p53 gene

The TSG *p53* is known to be mutated in approximately 70% of all known adult tumors.[3] In squamous cell carcinoma of head and neck region (SCCHN) 40–50% of the tumors studied have been a mutation in this gene.[5] The *p53* gene, so-called because it produces a 53 kDa nuclear phosphoprotein (wild type or normal p53 protein) is located on the short arm (p) of chromosome17.[8]

In normal cell biology, *p53* acts as a regulator of DNA synthesis.[3] The wild type (normal) *p53*, is essential for normal cell growth and the eventual suppression of the malignant phenotype. Inactivation of *p53* induces the development of malignancy. Thus, normal *p53* acts as a "molecular policeman," monitoring the integrity of the genome, usually residing in the nucleus. It is present at a very low concentration in all normal cells and tissues and has a very short half-life (6–20 min), so the wild type protein is almost undetectable in conventional immunohistochemical assays.[5,6,9]

Role of *p53* in normal cell cycle

The wild type p53 protein level strongly increases after DNA damage, and this is followed by a specific arrest of the cell cycle in the G1 phase. If DNA is damaged, wild type p53 accumulates and switches off replication to allow extra time for DNA repair. If the chromosome damage is too great, and the DNA repair fails, wild type p53 may trigger suicide by apoptosis.[6,3] However, tumor cells containing mutated or inactivated p53 are unable to induce this cell cycle arrest. Inactivation of p53 could increase on the other, the probability of their neoplastic transformation by inhibition of programmed cell death. Thus, p53 acts as a TSG in the normal form, but as an oncogene in its mutant form.[6]

The *p53* protein

The *p53* gene consists of 11 exons of which the first one is noncoding.[5] Exons 5–8 are the most highly conserved (codons 126–306), and contain the majority of mutations within the *p53* gene.[7] In turn, the p53 protein consists of 393 amino acids and comprises four regions with different functions.

The p53 protein has the ability to sense different kinds of stress to which cells are exposed, for example, DNA damage and ultraviolet (UV) irradiation. Once the p53 protein is activated after exposure to such stress it mobilizes a defence in which it acts as a conductor by activating other genes to produce proteins necessary in the defence process. In the currently accepted mode of p53 function, the inactive p53 protein, once activated by phosphorylation at specific residues, nonspecifically binds to DNA. The nonspecific binding is caused by the C-terminus of protein, leading to, and accordingly blocking, the central domain. This binding and blocking is, however, reversible by phosphorylation or acetylation within the C-terminus; and by reversing the blockade, binding of p53 becomes specific and p53 can thus act as a transcription factor.[5]

To perform its function, the p53 protein must, apart from being activated, be transported into the nucleus, and ideally also form tetrameric complexes with other p53 molecules. Following these events, the p53 protein may then induce growth arrest or cell death (apoptosis) – two powerful processes with devastating effects if uncontrolled or in the wrong environment. Being such a potent molecule, the normal levels of p53 protein in cells, as well as its activities, have to be regulated and kept under strict control. Several genes and their products are involved in this regulation.[5]

Activation of *p53*

So far, the activation of the *TP53* gene, the so-called upstream pathways, is not as well defined as the downstream pathways of the p53 protein that is the effect of p53 transactivation. However, today at least three genetically distinct pathways are known for the activation of the *TP53* gene, namely:

- Oncogenic stimuli through the alternate reading frame pathway

- UV irradiation and ionizing radiation through the ataxia telangiectasia mutated pathway
- Other stimuli, such as hypoxia, cytokines, and growth factors.

These stimuli cause the p53 protein to go through certain modifications; the type of modification seems to depend on and be specific to, the type of stress, species, and cell type. Different stimuli also activate the p53 protein through distinct pathways, so that p53 resulting from DNA damage is activated through phosphorylation and acetylation, whereas p53 resulting from oncogenic stimuli is "rescued" from degradation.[5]

Mutations at the *p53* locus in squamous cell carcinoma of head and neck

SCCHN is occasionally featured in the Li–Fraumeni syndrome, which is associated with germline mutations in the *TP53* gene. Germline *TP53* mutations have also been found in members of cancer-prone families and individuals with multiples tumors.[11]

Mutations in *p53* have shown to result from allelic loss, point mutation, deletion, or rearrangement. The wild type protein may also be inactivated by complex formation with mutant *p53*, viral aberrant host-binding proteins. These mutations result in either no expression of the wild type p53 or over expression of the mutant p53 protein. A direct correlation between abnormal p53 protein expression and gene mutation has been shown in SCCHN. A point mutation stabilizes the p53 protein and, together a loss of the normal gene, may lead to accumulation of the mutant protein within the cell. This would remove the normal function of the p53 and at the same time transform p53 into a dominantly acting oncogene.[6] Even in the absence of mutations, if allelic deletions are common, it is possible that cells harboring such deletions may have a growth advantage over their normal counterparts, favoring malignant transformations.[12]

Greenblatt *et al*., in their review of *p53* mutations and cancer, showed that in 524 cases of SCCHN, 31% of mutations were G: C→A: T transitions, 18% were G: C→T: A transversions, and 11% were G: C→C: G transversions.[13]

Yin *et al*., proposed a sequence of p53 alteration as follows:

a. *p53* mutations
b. Deletion of wild type allele
c. Increased dosage of mutated gene by aneuploid increase in chromosome copies, and/or
d. *p53* gene amplification.

Alteration in the *p53* gene is, therefore, a gradual process that spans many levels of tumor progression, and possibly involves four different molecular mechanisms.[6]

Recently, two new members of the *p53* family have become of interest, they are *p63* and *p73*.

p63 gene

The *p63* gene is located on chromosome 3q27–29 and expresses at least six different major transcripts. The molecular weights of the p63 protein range from 44 to 72 kDa.[5]

A direct role of p63 in tumorogenesis has not been demonstrated to date, although amplification of the 3q27 region has been detected in a number of tumors including squamous cell carcinoma. This is suggestive of a putative role of p63 as an oncogene rather than as a TSG.[5]

p73 gene

The *p73* gene has been considered a candidate TSG because of: (i) Its location in a region on chromosome 1p36.6 frequently deleted in certain tumors; (ii) its structural and functional homology with *p53*; (iii) its imprinting status; and (iv) its reduced expression in some tumors. However, its frequent mutation, biallelic expression and over expression in other tumor types contraindicate this hypothesis.[6] So far the well-characterized transcripts are p73α and p73β. When p73 protein is overproduced, it can activate transcription of p53-responsive genes and also induce apoptosis.[5] El-Naggar *et al*., in their study showed infrequent molecular alterations of the *p73* gene in SCCHN and that this gene plays a minor role in a subset of these tumors.[14]

Retinoblastoma gene

Rb is a human childhood disease, involving a tumor of the retina. It occurs both as a heritable trait and sporadically (by somatic mutation). The *Rb* gene is mapped on chromosome 13q14.[2] Rb arises when both copies of the *Rb* gene are activated. In the inherited form of the disease, one parental chromosome carries an alteration in this region. A somatic event in retinal cells that causes loss of the other copy of the *Rb* gene causes a tumor. In the sporadic form of the disease, the parental chromosomes are normal, and both *Rb* alleles are lost by (individual) somatic events. The cause of Rb is therefore loss of protein function, usually resulting from mutations that prevent gene expression (as opposed to point mutations that affect the function of the protein product) loss of Rb is involved also in other forms of cancer, including osteosarcomas and small lung cancers.[2]

Although Rb, and cyclin kinase inhibitors p16, p21, and p27 play a role in the cycle of a proliferating cell, the role that is relevant for tumorigenisis is more probably their function in the quiescent (G0) state. Loss of the *Rb* gene was said to be uncommon in SCCHN and oral carcinomas[11] (Maestro *et al*., 1996), however, in some reports lack of pRb expression has been observed in 66% of oral squamous

cell carcinomas (OSCCs) and 64% of premalignant lesions. *p16* expression is absent in 63% of OSCCs and 59% of the premalignant lesion.[16] Alteration in pRb/p16 expression is an early event in oral tumorigenisis and might be involved in the development of betel and tobacco related malignancies (Pande *et al*., 1998).[12] In contrast to this, Williams (2000) observed that pRb is strongly expressed in OSCCs, irrespective of differentiation.[16] Further studies are clearly necessary to elucidate its role in oral carcinogenesis.

doc-1

doc-1 gene is mutated in malignant oral keratinocytes, leading to a reduction of expression and protein function. Re-expression of *doc-1* in malignant oral keratinocytes results in the reversion of many malignant phenotypes back to normal, rendering the *doc-1*-transfected oral cancer cell to look like and act like its normal counterpart. The precise function of *doc-1* is normal oral keratinocyte biology is unclear. An 87 amino acid polypeptide that doc-1 shows a significant homology to a gene product induced in mouse fibroblasts by tumor necrosis factor–alpha (TNF-α). TNF-α decreases proliferation and increases differentiation. TNF-α is responsible for antiproliferation activity in human OSCC cell lines alone or in combination with interferon-α or -γ. It has been proposed that doc-1 may be an important regulator of TNF-α-induced keratinocyte differentiation/apoptosis.[3,16]

CYCLIN KINASE INHIBITORS

Cyclin dependent kinases (CDKs) and their regulatory partners, cyclins, form heterodimeric protein kinase complexes (each complex consists of a cyclin, a CDK, and proliferating cell nuclear antigen [PCNA]), that appear and degrade during predetermined steps in the cell cycle.[35] For example, the progression from the stationary G0 phase of the cell cycle through the G phase in mediated by two CDK complexes (CDK4 - cyclin D and CDK2 - cyclin E). The complexes are regulated by phosphorylation and dephosphorylation.[8]

In addition to regulation by cyclin binding and phosphorylation, CDKs are regulated by specific proteins called CDK inhibitors (CDKI). CDKI in mammals falls into two general families.[18]

1. The p21 family ($p21^{Cip1/WAF1}$, $p27^{Kip1,}$ and $p57^{Kip2}$)
2. The INK 4 family ($p15^{INK4b}$, $p15^{INK4a}$, $p18^{INK4c,}$ and $p19^{INK4d}$).

It is thought that $p21^{WAF1}$ mediates cell cycle arrest by inhibiting the CDKs that are required to drive the cell cycle. $p21^{WAF1}$ may also promote cyclin-CDK assembly. $p21^{WAF1}$ is localized in the nucleus, and forms a quaternary complex with cyclin A (or B, D, or E), CDK2 (or 4), and PCNA.[19]

A quaternary complex seems to be important for restraining the cell cycle in normal cells when CDKs complex with $p21^{WAF1}$, their kinase activity is inhibited. It is thought that the proliferating signal which drives the cell cycle, such as phosphorylation of Rb, activation of E2F, synthesis of DNA polymerase, and CDKs are not induced. Interestingly, the quaternary complexes have not always been detected in cancer cells. Their loss may contribute to the progression of cancer cells.[19]

Over expression of $p21^{WAF1}$ induces cell cycle arrest. Also, expression levels of $p21^{WAF1}$ are elevated in cells which are induced to arrest in G0–G1 phase by factors such as serum starvation, differentiation and senescence, X-ray, UV or DNA–damaging agents. Recent evidence has shown that $p21^{WAF1}$ can be induced through p53 – independent pathways by such various factors as TGF-β, TNF-α, Vitamin D, nerve growth factor, and okadaic acid.[19]

CYCLIN DEPENDENT KINASES 2A GENE (*P16*, MULTIPLE TUMOR SUPPRESSOR 1, CYCLIN DEPENDENT KINASE 4 1)

The existence of *p16* first became apparent from analysis of G1 cyclin-CDK immunoprecipitates, where it is found to be associated principally with cyclin D-CDK4.[20] The *CDKN2A* TSG is localized on chromosome 9p21. *p16* has been designated "multiple tumor suppressor 1" as well, since it is mutated in several cancers.[21] Genetic alterations involving the 9p21–22 region are common in human cancer, and the *CDKN2A* gene is considered to be the target in this region. Germline *CDKN2A* mutations have been shown to predispose to familial melanoma. SCCHN has also been in individuals from melanoma prone kindreds and germline *CDKN2A* mutations have been found. Somatic mutations of *CDKN2A* occur in 10% of SCCHN, and homozygous deletions occur in approximately 50% of cases. Methylation of *CDKN2A* is another important mechanism causing inactivation of this gene in SCCHN. It is thought that loss of 9p is an early event in the development of SCCHN and high frequencies of LOH at 9p21–22 are reported in dysplasia, carcinoma *in situ*, and SCCHN.[11]

p27 gene

The gene p27, a CDKI maps to chromosome 12p12–12p13.1.[38] Reduced levels of $p27^{Kip1}$ protein have been identified in a number of human cancers, and in some cases reduced $p27^{Kip1}$ expression is associated with an increased proliferative fraction. A study by Jordan *et al*., observed that $p27^{Kip1}$ protein was significantly reduced in oral dysplasias and carcinomas as compared with the normal epithelial controls. In addition, there was a significant reduction in $p27^{Kip}$ protein expression between low- and high-grade dysplasias,

suggesting that changes in $p27^{Kip}$ expression may be an early event in oral carcinogenesis.[11,21,26,29]

BRCA2 gene

BRCA2 is a TSG, which maps to chromosome 13q12–13 (Wooster *et al*., 1995). Germline mutations in this gene account for a large proportion of hereditary breast cancer families. An excessive number of SCCHN cases have been reported in *BRCA2* mutation carriers from several such families (Eastone *et al*., 1997). However, Kirkpatrick *et al*. (1997) found no mutations in the 16 tumors that were examined for mutations in the coding exons of *BRCA2*. Nawroz-Danish *et al*. (1998) also did not find abnormalities in 37 tumors they analyzed for exon 11 of *BRCA2* at the transcriptional and translational levels for truncation mutations. Hamel *et al*. (1999) suggest that neither somatic *BRCA2* mutations in tumors, nor frequent germline *BRCA2* mutations are associated with head and neck cancer development. The results imply that it is unlikely that *BRCA2* is the putative 13q TSG associated with SCCHN development.[22,23]

Fragile histidine triad gene

Fragile histidine triad (*FHIT*) is a TSG mapped to chromosome 3p14.2. It encodes the *FHIT* protein which has a dinucleoside triphosphate hydrolase activity. Various investigators have suggested that the *FHIT* gene is altered in SCCHN with decreased or aberrant protein but no mutations or deletions.[7] Loss of function of the protein may be important in the development and/or progression of head and neck cancer (Corce *et al*., 1999).[11] In the absence of the *FHIT* protein, di-adenosine-tetraphosphate may accumulate, leading to DNA synthesis and cell replication.[24]

E-cadherin gene

E-cadherin is one of the most important molecules in cell-cell adhesion in epithelial tissues. It is localized on the surface of epithelial cells in regions of cell-cell contact known as adherens junction. Classical cadherins, E, and N-cadherins being the best characterized play important roles in the formation of tissues during gastrulation, neurulation, and organogenesis.[14]

The human epithelial (E)-cadherin gene maps to chromosome 16q 22.1. It encodes a 120 kDa glycoprotein with a large extracellular domain, a single transmembrane segment and a short cytoplasmic domain, which interacts with the actin cytoskeleton through linker molecules, α-, β-, and γ-catenins.

Besides its role in normal cells, this highly conserved gene can play a major role in malignant transformation, and especially in tumor development and progression. The suppression of E-cadherin expression is regarded as one of the main molecular events responsible for dysfunction in the cell to cell adhesion. Most tumors have abnormal cellular architecture, and loss of tissue integrity can lead to local invasion. Thus, loss of function of E-cadherin tumor suppressor protein correlates with increased invasiveness and metastasis of tumors, resulting in it being referred to as the "suppressor of invasion" gene.[14]

LOH on 16q is detected frequently in metastasizing malignancies derived from the breast, esophageal, pulmonary tumors, and SCCHN. In SCCHN, loss of expression has been correlated with a high-grade and an advanced stage of the disorder, with poor prognosis (Saito *et al*., 1999).[7]

Adenomatous polyposis coli gene

Adenomatous polyposis coli (*APC*) gene is a TSG found on chromosome 5q21. The *APC* gene is a TSG because of the association between mutations or LOH at the APC locus of chromosome 5q21 and colorectal cancers and because of inherited mutations in APC result in familial cancers.[25,30,31,35,36]

LOH with mutation of the *APC* TSG has not been detected frequently in oral cancers, although Huang *et al*. in their study found a 53.8% LOH.[25] Recently, it has been shown that the APC protein might indirectly regulate the E-cadherin-catenin complex because in E- cadherin negative colon carcinoma cell lines, β-catenin is preferentially bound to APC protein. If, however, these cell lines are transfected with E-cadherin, β-catenin redistributes from the APC bound complex to the E-cadherin-catenin complex and is accompanied by growth inhibition and decreased tumorigenicity. At present, however, it is unknown whether APC protein controls the E-cadherin-catenin complex in oral carcinoma.[16,40,41]

CONCLUSION

Oral cancer is a particularly challenging pathology. Advances in diagnosis and treatment have slowly accumulated, but a sound understanding of underlying cell biology is likely to enable further, much needed progress. TSGs, or more precisely, the proteins they code for, either have a dampening or repressive effect on the regulation of the cell cycle or promote apoptosis, and sometimes do both. The functions of tumor suppressor proteins fall into several categories which influence the overall pathology of oral cancer. Knowing in depth about these TSGs will definitely help in diagnosis and treatment of oral cancer and lead to better prognosis.

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