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Molecular Mechanisms of Head and Neck Cancer

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Abstract

Background—With a global incidence rank of eight and a significant portion of head and neck malignancies (~90%), oral squamous cell carcinoma (OSCC) poses a major health risk and is one of the leading cause of mortality in developing nations [1]. Distribution of the incidence of OSCC varies across the world with south-central Asia and Africa leading, followed by eastern and central Europe, and to a lesser extent Australia, Japan, and the United States. Over the past few years there has been a drastic increase in the incidence of oral cancer in most parts of the world [2]. In the United States alone, with about 17 new cases/100,000, oral cancer is the fifth most common and sixth leading cause of cancer-related mortality per year [3]. While men tend to have a higher incidence of OSCC (6.6/100,000) compared to women (2.9/100,000), there is an equal mortality rate between the sexes (50%).

Standard of care

Diagnosis

Early diagnosis and treatment of precancerous lesions, which tend to last for many years before advancing to a highly malignant metastatic form, has been shown to result in an increased 5-year survival rate. In spite of the advances in the diagnostics and therapeutics, specially of early stage disease (stages I and II), the overall 5-year survival rate for oral cancer remains at about 50% [35,36]. This has been attributed to either identification of lesions at advanced stages of the disease, or, metastases and/or second tumors arising in patients following resection or treatment of the primary tumor.

Significant advances have been made in recent years in diagnosis and treatment of local primary OSCC tumors. Diagnoses techniques include the use of Toluidine Blue, which stains the nuclei of rapidly dividing cells in malignant lesions dark blue, and indirect mirror examination of the oral, nasopharyngeal and laryngopharyngeal regions, Photodynamic diagnosis using 5-aminolevulinic acid (5-ALA) [37,38], autofluorescence, using flavin based staining for healthy tissue, and CT scans are routinely used to identify extent of spread of the primary tumor as well as possible metastases [39].

Treatment

Depending on the stage and extent of disease, treatment options for OSCC include surgery, radiotherapy, chemotherapy, and various combinations of these. Treatment options for OSCC are selected based on numerous factors including stage, grade and location of the tumor and, health and age of the patient. Newer treatment options include immunotherapy and gene therapy, both of which are in the initial stages of validation. Numerous molecular targets are also being explored for developing drugs for treatment of OSCC. These approaches are preferred over surgical resection, a standard approach for treatment of OSCC tumors, specially since it leaves the patient disfigured and in need for follow up reconstructive surgery. Improvements in treatment of primary OSCC have been achieved with combination therapy,

wherein radiation and chemotherapy have been combined, either together, or with molecular-targeted therapies. These together have improved 3-year survival rates from ~15% to 35–50%. Combinations of radiation and chemotherapy (using taxanes) have increased survival rates to about 70% [40].

Molecular Genetics

Understanding the molecular and genetic alterations in the pathogenesis of OSCC will help elucidate the mechanisms involved in tumor formation as well as identify potential targets for improved treatment of OSCC. OSCC formation occurs through accumulation of multiple genetic alterations. OSCC formation, which is a multistep process, is initiated by chemical insults to the normal oral epithelium by carcinogens including tobacco, alcohol, etc. This results in genetic alterations and formation of preneoplastic lesions and eventually OSCC. These changes are characterized by increasing genetic instability, aneuploidy, increased dysplasia, altered expression of cell surface markers, loss of cellular organization, and eventually invasive penetration through the basement membrane. Though multiple pathways, which promote cell proliferation, cell survival, and/or transformation capabilities, have been identified understanding of molecular pathway cooperativity involved in OSCC tumorigenesis is still lacking.

Etiology and risk factors

Oral cancer includes the tumors localized to the oral cavity and includes tumors arising on the tongue, buccal mucosa, lips, hard and soft palate, gums and the base of the mouth [4]. OSCC is frequently associated with high risk behaviours, including, smoking, alcohol consumption, and the use of smokeless tobacco. OSCC risk is additive and has been shown to be higher in patients with a history of alcoholism and smoking compared to normal population and to independent effects of smoking or alcohol [5–7].

Additionally, smokeless tobacco usage, including betel leaves, miang, qat, etc., has been shown to cause and/or promote OSCC tumorigenesis.

Human papilloma virus (HPV) has been associated with the genesis of OSCC, specifically a subset of tumors which are localized to the lingual and palatine tonsils within the oropharynx [8]. HPV16 infections have been associated with a 3–6 fold increase in oropharyngeal OSCC risk [9–14]. OSCC incidence arising from HPV infections combined with alcohol or tobacco abuse has not been established due to limitations in sample sizes.

Dietary intake has been shown to have the most significant effect on OSCC compared to any other malignancy. A diet rich in fruits and vegetables has been shown to reduce the risk of OSCC, in patients with and without a history of tobacco and/or alcohol abuse [6,15–22].

Although OSCC is rarely associated with inherited cancer syndromes, a few heritable disorders have been shown to increase the risk of an individual to OSCC. The tumors have a younger age of onset, typically, below 40 years. OSCC have been reported in patients with Fanconi anemia. These patients, who inherit recessive autosomal mutations in genes regulating DNA recognition and repair, show an increased risk of bone marrow failure, leukemia, and solid tumors including OSCC. Fanconi anemia patients have a 500- to 700- fold increased risk of OSCC formation [23–26]. Increased risk of OSCC has also been reported in individuals inheriting functionally inactivating mutations in the CDKN2A gene. Somatic inactivation of this important regulator of cell cycle at the G1-S boundary has also been reported in sporadic OSCC tumors [27–30]. The case for the presence of predisposing genetic factors for OSCC is strengthened by the fact that positive family history increases an individual's risk for OSCC

by 2- to 4- fold. This risk is greater if the affected family member is a sibling (8-fold or more). The risk of second tumors is also higher in these individuals [31–34].

Chemical carcinogenesis and the Ras pathway

As with most models of epithelial tumorigenesis, chemical carcinogens have been used to induce OSCC formation. The most frequently used inducers include, 9,10-dimethyl-1,2-benzanthracene (DMBA) and 4-nitroquinoline-1-oxide (4NQO) [41–43]. The administration of 4NQO through drinking water or via local application in experimental animals has been shown to faithfully replicate the human disease progression from preneoplastic lesions to fully blown tumors. And similar to the human disease, this model induces hyperplasia and dysplastic lesions with long-term administration of small doses of carcinogen. Application of the 4NQO has been shown to result in polyploidy, activation of oncogenes, including Ha-Ras, and loss of function for tumor suppressor genes, including p53, and cell adhesion molecules, E- and P-cadherins [44–47].

Genetic alteration in preneoplastic lesions

A few genomic regions of loss have been implicated as early events in the formation of preneoplastic lesions. These include regions on chromosomal arms 9p, 17p and 3p. The genes involved in early genesis of OSCC, which map to these regions of frequent loss, include *p14^{ARF}* and *p16^{INK4a}* on 9p21, TP53 on 17p13, and FHIT on chromosome 3p14. Deletions of 4q, 6p, 8p, 13q, and 14q and amplification of 7p and 11q have also been reported in OSCC at later stages of tumor formation [48–52].

p16^{INK4a}/p14^{ARF} locus—The p16 gene is involved in the regulation of the RB-pathway. p16 inhibits the activity of Cyclin D/CDK-4, -6 complexes to prevent the phosphorylation of pRB, and hence preventing cell cycle progression. Product of the alternate transcript, *p14^{ARF}*, interacts with MDM2, which is involved in degradation of TP53. Loss of the INK/ARF locus results in deregulation of both, p53 and pRB, pathways and hence uncontrolled cell proliferation [53].

TP53—Normal TP53 function is lost in almost all tumors, either through mutations or genomic deletions of the gene, or through up-regulation of negative regulators of TP53 function. Loss of heterozygosity for the TP53 locus has been reported in about 60% of OSCC [54–57]. It is almost impossible to detect TP53 expression in normal tissue due to a high turnover of the TP53 protein. This expression pattern of TP53 is altered in tumor tissue wherein mutant forms of TP53, which are highly stable, are readily detected by immunohistochemical analyses. Approximately 50% OSCC tumors stain positive for TP53 [58,59]. Another member of the p53 family implicated in OSCC genesis and progression is P63. P63 is thought to play a role in differentiation of keratinocytes and epithelial cells. Decreased P63 expression in OSCC, in the context of TP53 expression, has been shown to correlate with increased metastatic load and decreased overall survival [59].

Cyclin D1—CDK4/6-Cyclin D complexes are involved in phosphorylation of pRB, a major regulator of the G1/S transition of cell cycle. Phosphorylation of pRB results in release of bound E2F transcription factors, premature transition into S-phase, and, a resultant non-diploidy of DNA. Amplification of the Cyclin D1 locus, 11q13, has been reported in numerous epithelial malignancies. Approximately 30–50% of OSCC tumors show an amplification and/or increased protein expression [60–62].

EGFR and EGFR signaling—Another region of amplification is 7p12. This amplicon encodes the EGFR gene that, along with its ligand Transforming growth factor alpha (TGF- α), has been shown to be amplified and overexpressed in 80–90% of OSCC tumors [63–66].

In addition, EGFR signaling has been shown to promote metastases [63]. High EGFR expression has been shown to be present even in 'healthy' tissues surrounding the tumor lesions in cancer patients compared to normal population [67].

EGFR encodes a transmembrane receptor that is activated by its ligands EGF and TGF- α . Activation of EGFR by its ligands results in homodimerization of the receptor and subsequent activation of tyrosine kinase via the intracellular domain of the receptor. This activates the downstream MAP kinase signaling cascade and promotes cell proliferation and angiogenesis, and inhibits apoptosis. Phosphorylation mediated activation of MEK and ERK proteins results in their translocation into the nucleus and activation of a number of transcription factors which control cell proliferation, migration and differentiation [68,69].

TGF- β pathway—The TGF- β pathway has been shown to play a significant role during embryogenesis, organogenesis, and in tumor formation, specially in epithelial tumorigenesis. These effects of the TGF- β pathway have been attributed to the positive, as well as negative effects of TGF- β and its down stream effector molecules on cell proliferation, survival, and differentiation. The TGF- β cytokines signal predominantly through the serine-threonine kinase family of receptors, TGF- β receptors type I and type II (T β RI and T β RII, respectively), and downstream effector molecules including SMAD proteins -2, -3, -4, and -7. Alterations in expression or activity of individual members of the TGF- β pathway has been detected not only in multiple epithelial tumors, but also in tumors influenced by their epithelial environment. These include carcinomas of the breast, prostate, gastrointestinal tract (stomach, colon, and rectum), lung, cervix, liver, and skin. Although mutations of the individual members of the TGF- β pathway have been identified in OSCC and other epithelial tumors, they are relatively rare [70–74]. A more frequent event is a reduction in the expression of T β RII [75]. This is evident in knockout mouse models where there is an increase in the penetrance and rate of tumor formation, and metastases in various tissues [76–84]. T β RII expression has been shown to be affected by: a) mutations in the promoter region of the gene [85,86]; b) activated oncogene activity, including Cyclin D1, and Ras [87,88]; c) epigenetic regulation [89]. Thus, signaling via the T β RII has been suggested to have tumor suppressive function in the tumorigenic process. Reduced expression and hence attenuation of T β RII signaling will result in increased resistance to TGF- β mediated growth suppression, increased cell proliferation, decreased differentiation and increased transformation capacity of the tumor cells [87,90,91].

In oral cancer, the above observations hold true, with a majority of OSCC tumors, both primary and second, exhibiting reduced expression of T β RII, and only a few tumors showing mutations in the members of the TGF- β pathway [92]. Numerous studies have shown that most primary oral squamous cell carcinomas retain TGF- β signaling. This signaling diminishes as the disease progresses from carcinoma to metastases. During this transition a significant decrease in T β RII expression, resulting from down-regulation of gene expression and not mutations, has been observed [75]. Understanding the effects of this decrease in TGF- β signaling and identifying the molecules involved in modulation of cell cycle, proliferation, survival and differentiation in response to TGF- β will help in developing novel targeted therapies for treatment of oral cancer.

Multiple chromosomal abnormalities have been reported in OSCC by cytogenetic and spectral karyotype analyses. Frequent loss of heterozygosity has been reported for chromosome 18 in OSCC implicating SMAD-2 and -4 as important players in the tumorigenic process [93]. Mutations, though relatively rare, have also been identified in the SMAD genes and other regulators of the TGF- β pathway in primary OSCC tumors and cell lines [75,92,94–97].

CDK2AP1—Also known as *p12^{CDK2AP1}* and Deleted in Oral Cancer -1 (DOC-1), this gene was identified by subtractive hybridization from a hamster oral cancer model [98]. CDK2AP1,

a novel cell cycle regulator, has been shown to regulate cell cycle by interacting with CDK2 and preventing phosphorylation of pRB, thus rendering pRB active and able to regulate cell cycle [99]. CDK2AP1 has also been shown to interact with DNA polymerase- α /primase and reduce its activity [100]. This interaction affects the initiation, but not elongation step of DNA replication. Although an exact mechanism is not known, it is hypothesized that CDK2AP1 inhibits DNA polymerase- α /primase activity either through direct interaction or through its interaction with CDK2. Reduced or absent expression of CDK2AP1 has been demonstrated in about 60% of human oral cancer cell lines and tumor samples [101–103]. CDK2AP1 is also a downstream target for the TGF- β pathway. There is a significant correlation of reduced T β RII expression and CDK2AP1 expression [104]. Thus, CDK2AP1 is a common link between two pathways, the pRB pathway and the TGF- β pathway, both of which are affected in the process of OSCC tumorigenesis. Figueiredo *et al* explored this advantage to use recombinant DNA to re-express CDK2AP1 in OSCC tumors in murine models and showed successful tumor regression [105].

Angiogenesis and vasculogenesis—Blood vessel formation is a critical step in providing nutritional support for tumor growth. Vascular Endothelial Growth Factor (VEGF) is a potent inducer of blood vessel formation and is upregulated in almost all tumors. 10–20% of dysplastic oral lesions progress to an invasive phenotype. This is accompanied by increase in blood vessel formation and increased blood supply. Increased expression of VEGF is seen in OSCC through all stages of disease progression [106–108]. Other factors that promote blood vessel formation and endothelial cell migration include, prostaglandin E₂ (PGE₂) and TGF- β are often overexpressed by tumor cells [109–113]. COX-2 inhibition has been shown to be effective in controlling OSCC cell proliferation and this is shown to be mediated specifically through the inhibition of PGE₂.

In addition to angiogenesis, the embryonic phenomenon of vasculogenesis has also been described in the OSCC tumorigenic process. Pak and coworkers have demonstrated an increase in the number of CD34⁺ progenitor cells in human OSCC tumors [114]. These cells have a capacity to develop into cells that contribute to the tumor vasculature [115,116].

Other genes—p27^{KIP} is a negative regulator of G1/S transition and functions to regulate cell cycle progression by sequestration and inhibition of the Cyclin D/CDK-4, -6 complex. p27 expression also associated with the differentiation status of OSCC tumors with well-differentiated tumors showing positive reactivity for p27 and less differentiated tumors showing low or no p27 expression. Expression of p27 is downregulated early in OSCC [117, 118]. E-Cadherin, a membrane associated protein, is required for contact inhibition of cell proliferation and cell growth. Loss of E-Cadherin expression due to promoter hypermethylation has been correlated with poor prognoses of oral cancer patients [119–121].

Expert commentary

Although progress has been made in the areas of diagnostics and therapeutics of OSCC, second tumors and metastases still remain a cause for low 5-year survival and high rates of morbidity and mortality. Combination radiation and chemotherapy approaches have seen improvement in the overall survival of stage I and II patients, but satisfactory treatment options for advanced disease still remain to be achieved. Thus, there is a desperate need for new approaches in cancer treatment. Gene therapy strategies are being tested and in combination with chemotherapy and radiation therapy will prove more powerful. Gene therapy approaches are already being tested using adenovirus mediated p53 re-expression, liposome mediated CDK2AP1 re-expression, and antisense inhibition of EGFR [63,105,122]. Inhibitors of specific molecular targets upregulated in the tumorigenic process (EGFR, VEGF, COX-2) are also being tested, either alone or in combination with existing therapies. Trials are ongoing for combination therapies

in both the US and in Europe. Unless the efficacy of these novel therapeutic approaches is tested and proven in a large cohort, the surgery, chemotherapy and radiation remain as the only options to treat OSCC.

Five-year Review

A large number of resources are being invested in development of therapies for OSCC. As our understanding of the intersecting nodes of altered molecular pathways involved in OSCC genesis improves, we believe that therapeutic approach for this disease in the future will be more direct and focused. Thus as our understanding of the fundamental molecular changes improves, so will our ability to treat the disease.

Key Issues

Oral squamous cell carcinoma is a significant worldwide health burden with about 500,000 cases annually

Second tumors and metastases are the most frequent causes of high mortality in OSCC patients.

Chemotherapy and radiation therapy, only second to surgery, are the current treatment options but are successful only with stage I and II OSCC.

Targeted inhibitors of EGFR and VEGF signaling are currently being tested in clinical trials for treatment of OSCC, but more advances in treatment options and identification of better targets is required.

Better understanding of intersecting nodes of various molecular pathways altered in OSCC is needed to improve target identification.

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