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A Narrative Review on the Genetic and Molecular Basis of Oral Cancer: Unraveling Complexity and Significance

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The process of oral carcinogenesis involves multiple molecular and histological stages characterized by genetic and phenotypic molecular markers. This process entails the upregulation of various proto-oncogenes and oncogenes, as well as the inactivation of tumor suppressor genes. Consequently, there is increased activity of growth factors and their receptors on the cell surface, leading to amplified intracellular signaling and enhanced production of transcription factors. It is important to note that oncogenes alone do not drive carcinogenesis; rather, genes with tumor suppressor activity play a crucial role in inducing phenotypic changes in cells, resulting in increased cell proliferation, loss of cellular cohesion, and the ability to invade local tissues and metastasize to distant sites. A comprehensive understanding of the molecular interactions between oncogenes and tumor suppressor genes can facilitate more precise diagnosis and prognosis assessment, potentially paving the way for innovative treatment strategies.

Keywords: Moral carcinogenesis, genome instability, mutation, tumor-promoting inflammation.

1. Introduction

Oral squamous cell carcinoma ranks as the sixth most prevalent cancer worldwide and is the most common cancer in India, accounting for a significant portion of total cancer-related mortality(1). It primarily affects various regions of the oral cavity, including the anterior tongue, cheek, floor of the mouth, retromolar area, gingiva, or buccal mucosa. During carcinogenesis, multiple genetic events disrupt the normal functions of both oncogenes and tumor suppressor genes. However, the relative importance of these known gene alterations remains unclear and is not yet fully elucidated.(2)

It is widely recognized that a combination of environmental and genetic factors influences the

complex process of carcinogenesis. Genetic events disrupt the normal regulatory mechanisms controlling fundamental cellular functions such as cell division, differentiation, and apoptosis (3). Boyd and Reade (1988) outlined three primary mechanisms involved in the carcinogenesis of the oral mucosa: chemical, physical, and viral mechanisms (4). Subsequently, Hanahan and Weinberg (2000) identified six hallmark characteristics of cancer, including the acquisition of growth signaling autonomy (through oncogenes), loss of growth-inhibitory signals (involving tumor suppressor genes), evasion of apoptosis, cellular immortalization, angiogenesis, and invasion/metastasis(5). A subsequent review (referred to as hallmarks II) added two emerging hallmarks: reprogramming energy metabolism and evading immune response, along with two enabling traits: genome instability and mutation, and tumor-promoting inflammation(6).

The histologic progression of oral carcinogenesis, from hyperplasia to dysplasia, severe dysplasia, and eventual invasion and metastasis, is thought to result from the accumulation of these genetic changes. Genetic alterations during carcinogenesis may manifest as point mutations, amplifications, rearrangements, or deletions.(7)

The genetic theory of cancer

Changes in regulatory pathways occur during the development of cancer.

Oral carcinogenesis is a complex process involving genetic alterations in signal transduction pathways that regulate normal cellular functions. In normal conditions, oral epithelial cell biology is tightly regulated by both excitatory and inhibitory pathways controlling cell division, differentiation, and senescence. These pathways involve binding extracellular ligands to cell surface receptors, initiating signals that are transmitted intracellularly to affect cell function or gene transcription(8). However, in oral cancer, there is an accumulation of changes in these cellular signals, occurring at various levels of the pathway. Oral epithelial cells acquire these mutations, becoming functionally independent from surrounding normal cells. Consequently, tumor cells exhibit rapid proliferation, angiogenesis, alterations in signalling, and invasive behaviour.(8)

Oncogenes and tumor suppressor genes are crucial regulators of cellular growth, normally expressed in cells to maintain normal function. Dysregulation or inappropriate expression of these genes can lead to the development of neoplasia. Genetic alterations affecting these genes in cancer cells can be classified into two types: dominant type, involving proto-oncogenes and oncogenes which result in a gain of function, and recessive type, involving tumor suppressor genes, growth suppressor genes, recessive oncogenes, or anti-oncogenes, which result in a loss of function.(9)

The hallmark feature of cancer is characterized by rapid and uncontrolled cell growth, a process heavily influenced by the dysregulation of cell cycle regulatory molecules. In the context of head and neck cancers, the pathogenesis involves key players such as the cyclin-CDK complex and the retinoblastoma protein (RB)(10). Phosphorylation of RB by the cyclin/CDK complex results in the release of E2F, which in turn initiates the transcription of genes necessary for the progression through the G1/S transition of the cell cycle. Specifically, the RB function is modulated by the activity of cyclin E/CDK2. Conversely, CDK4 and CDK6 act upstream of RB, inhibiting its function through phosphorylation(11).

In head and neck cancers, alterations in RB function, whether upregulation or downregulation,

have been observed, contributing to varying degrees of malignancy and aggressiveness depending on the cellular context. Downregulation of RB function allows the cell cycle to proceed unchecked, leading to continuous cell division and proliferation. On the other hand, upregulation of RB function results in a decrease in pro-apoptotic signals that are normally triggered during the cell cycle. In either scenario, disruptions in the RB pathway significantly impact cell-cycle progression, promoting cancer cell survival.

Oncogenes and oncoprotein

Oncogenes are categorized based on the functions of their normal counterparts, known as proto-oncogenes, in the biochemical pathways regulating cellular growth and differentiation. These categories encompass various aspects of cellular physiology:

1. Growth factors: Oncogenes associated with growth factors like Transforming Growth Factor (TGF), Fibroblast Growth Factor (FGF), and Platelet-Derived Growth Factor (PDGF).

2. Cell surface receptors: This category includes oncogenes related to cell surface receptors such as the Epidermal Growth Factor Receptor (EGFR) and Fibroblast Growth Factor Receptor (FGFR).

3. Intracellular signal transduction pathways: Oncogenes involved in intracellular signal transduction pathways, such as the RAS oncogene.

4. DNA binding nuclear proteins transcription factors: Oncogenes in this category include transcription factors like MYC, FOS, and JUN, which regulate gene expression within the nucleus.

5. Cell cycle proteins: This category encompasses oncogenes related to cell cycle regulation, including cyclins and cyclin-dependent protein kinases (CDKs).

6. Inhibitors of apoptosis: Oncogenes involved in inhibiting apoptosis, such as bcl-2, which prevent programmed cell death(12).

Oncogenes are defined as altered growth-promoting regulatory genes or proto-oncogenes that govern the cell's signal transduction pathways. Initially discovered in retroviruses causing cancers in birds and cats, oncogenes result from alterations or mutations in proto-oncogenes, leading to overproduction or gain-of-function changes in these regulatory proteins. While oncogenes alone cannot transform a normal oral keratinocyte into a malignant one, they serve as initiators of the tumorigenesis process(13).

Aberrant expression of several oncogenes plays a crucial role in the development of oral carcinogenesis, including proto-oncogenes like Epidermal Growth Factor Receptor (EGFR/c-erb 1), members of the ras gene family, c-myc, int-2, hst-1, PRAD-1, and bcl-1.

Proto-oncogenes have the potential to participate in tumorigenesis due to their roles as relays in the complex biochemical circuitry governing vertebrate cell phenotype, including signaling from cell surface hormones, receptors, intracellular signal transduction proteins, and nuclear factors orchestrating genetic responses.

Proto-oncogenes act through three main biochemical mechanisms(14)-

1. Phosphorylation of proteins, involving substrates such as serine, threonine, and tyrosine.

2. Transmission of signals by GTPases, exemplified by the RAS oncogenes' discovery, encoding a previously unknown variety of GTPase.

3. Control of transcription from DNA, involving a growing variety of transcription factors like FOS and MYC, which also participate in DNA replication.

Growth factor receptors and mechanisms

Activation of growth factor receptors in human tumors can occur through various mechanisms, including mutations, gene rearrangements, and overexpression. Several signalling pathways implicated in both cancer and stem cell development include the JAK/STAT pathway, NOTCH signaling pathway, MAP-Kinase/ERK pathway, PI3K/AKT pathway, NFkB pathway, Wnt pathway, and TGFβ pathways(15).

In normal growth factor receptors, the kinase is transiently activated upon binding of the growth factor ligand to the receptor, leading to rapid receptor dimerization and tyrosine phosphorylation of multiple substrates involved in the signaling cascade. However, oncogenic growth factor receptors can cause dimerization and activation independent of specific growth factor ligand binding, resulting in continuous mitogenic signals to the cell.

In oral carcinogenesis, deregulation of growth factor receptors often occurs through increased production and autocrine stimulation. Aberrant expression of transforming growth factor alpha (TGF-α) and beta (TGF-β) is observed in carcinogenesis. TGF-α acts in association with EGFR, while TGF-β follows a pathway involving SMAD2 and SMAD3. TGF-α is reported to be early in oral carcinogenesis, stimulating cell proliferation by binding to EGFR and promoting angiogenesis. It is also found in "normal" oral mucosa of patients who later develop a second primary carcinoma, suggesting a premalignant lesion with rapid proliferation and genetic instability.

Patients with oral tumors overexpressing both TGF-α and EGFR have been shown to have significantly shorter survival than those overexpressing EGFR alone. TGFβ1 signals through TGFβ receptors, which phosphorylate SMAD2 and SMAD3 along with SMAD4 to regulate the transcription of target genes.

Recently, a connection between the TGFβ signaling pathway and nuclear factor-κB (NF-κB) has been studied. NF-κB is a transcription factor providing an important survival signal to cells. Abrogation of the TGF-β pathway has been associated with NF-κB activation, suggesting a link between decreased TGFβ signaling and NF-κB activation.

Cell surface receptors

The binding of cell surface receptors with ligands initiates the translation of signals from the extracellular environment through the cell membrane, activating a cascade of biochemical reactions. Mutations or amplifications in genes encoding growth factor receptors can lead to an increased number of receptors or the production of continuous ligand-independent mitogenic signals. EGFR, a 170,000-Da phosphoglycoprotein, is considered a significant oncoprotein in oral cancer(16).

Three mechanisms have been proposed to activate the EGFR gene in carcinogenesis:

1. Deletion or mutations in the N-terminal ligand-binding domain.

2. Overexpression of the EGFR gene concurrent with continuous presence of EGF or TGF-α.

3. Deletion in the C-terminus of the receptor prevents downregulation of the receptor after ligand binding.

In human oral carcinogenesis, EGFR is overexpressed due to gene amplification. Consequently, malignant oral keratinocytes possess 5–50 times more EGF receptors compared to their normal counterparts. The mechanism of signal transduction in oral carcinogenesis, whether due to overexpression of normal receptors resulting from mutated genes or the formation of new receptors, is not yet fully understood. Oral tumors with EGFR overexpression have been observed to exhibit a higher response to chemotherapy compared to EGFR-negative tumors, likely due to their higher intrinsic proliferative activity leading to increased sensitivity to cytotoxic drugs(17).

Intracellular signal transduction pathways (RAS)

Similar to growth factor receptors, intracellular messengers can undergo intrinsic activation, delivering a continuous signal rather than one regulated by ligands. Oncogenes can be activated through gene amplification and/or mutation. In oral squamous cell carcinoma (OSCC), the ras oncogene is frequently genetically altered, with mutations in three isoforms - Hras, Kras, and Nras - producing the same phenotype in vitro transformation assays. Mutations in Hras appear to be highly prevalent in OSCC compared to Kras and Nras, which have been reported approximately in the range of 0 to 55%.

Mechanism of ras activation:

These genes encode closely related proteins located on the cytoplasmic side of the cell membrane, transmitting messages from cell surface receptors to intracellular regulatory enzymes. RAS proteins on the cytoplasmic side of the cell membrane are activated by growth factors through enhanced exchange of guanine nucleotide, forming the Grb2 SOS complex. The molecular mechanism underlying ras activation depends on the whole superfamily of small G-proteins, as there exists a switch between the GTP-bound active and GDP-bound inactive state(18).

In normal human cells, an equilibrium is strictly maintained by the activity of GAPs (GTPase activating proteins) and GEFs (Guanine nucleotide exchange factors) between the active and inactive states, as ras proteins have minimal measurable activity on their own. GAPs accelerate the GTP hydrolysis of ras, while antagonist GEFs such as ras-GRFs and ras-GRPs catalyze and weaken GDP replacement with GTP. In cells where ras is mutated, the equilibrium between the GTP and GDP-bound states is impaired. Ras mutations predominantly occur at codons G12, G13, and Q61 in K-RAS and H-RAS. Point mutations in K-RAS and H-RAS lead to GAP-catalyzed hydrolysis of GTP to GDP, generating constantly active ras, responsible for downstream effector activation leading to aberrant cell functioning and malignancy.

Ras and its major signalling pathways:

Ras oncogenes are associated with proteins involved in the transduction of extracellular growth, differentiation, and survival signals. Ras activates receptor tyrosine kinases (RTKs), which in turn activate two key signal transduction components: Small GTPase and lipid kinase

PI(3)K. Activated ras stimulates the mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3-kinase (PI3K)/Akt pathways. Downstream steps involve phosphorylation by RAF1 kinase on two distinct serine residues MEK1/2. MEK1/2 further phosphorylates specific threonine and tyrosine residues in the activation loops of ERK1/2, leading to growth and differentiation. Ras also transduces the PI3K/Akt signalling pathway, promoting cell cycle proliferation and survival.

DNA binding nuclear proteins transcription factors (MYC, FOS, JUN)

Transcription factors, proteins responsible for activating other genes, undergo alterations in oral cancer. A growing number of known proto-oncogenes encode nuclear proteins, regulated by receptor-activated second messenger pathways. Inactivation of these genes leads to cell cycle arrest, preventing mitogenic and differentiation responses to growth factors.

The c-myc gene, involved in regulating cell proliferation and apoptosis, is frequently overexpressed in oral cancers due to gene amplification. It's notably found in poorly differentiated tumors, but recent studies show its overexpression in moderate and well-differentiated oral carcinomas as well, where cell proliferation outweighs apoptosis. C-myc interacts with the retinoblastoma tumor suppressor gene Rb-1 nuclear protein pR6, inhibiting cell proliferation. However, phosphorylation of pR6 increases c-Myc levels, promoting cell proliferation. Another amplified transcription factor in head and neck cancers is PRAD1 (also known as CCND1 or cyclin D1), functioning as a cell cycle promoter(15).

There's no specific order of oncogene activation in oral cancers; instead, the accumulation of activated oncogenes appears to be crucial. The significance of identified oncoproteins in oral carcinogenesis is under investigation. Other oncogenes linked to oral cancer development include hst-1, k-2, bcl-1, sea, men-1, and em1s-1. However, oncogenes alone are not sufficient for oral cancer development but serve as initiators, working in conjunction with the inactivation of tumor suppressor genes. The critical event in transforming a "premalignant" cell into a malignant one is the inactivation of cellular negative regulators, tumor suppressor genes.

Cell cycle proteins (cyclins and cyclin-dependent protein kinases)

The cell cycle regulates mammalian cell proliferation and comprises four functional phases:

a. S phase (DNA replication)

b. G2 phase (preparation for mitosis)

c. M phase (division of DNA and cellular components into two daughter cells)

d. G1 phase (commitment and preparation for another round of replication)

S and M phases are crucial processes in all cell cycles for cell replication, requiring the coordinated expression of cyclins and cyclin-dependent kinases (Cdks) in response to growth factors.

Cdks, including Cdk2 and Cdk1, play essential roles in directing S and G2 phase transitions, with Cdk1 governing the G2/M transition and mitotic progression(19). Cdks can be categorized into two groups:

a. 'Cell cycle' Cdks, which regulate cell cycle progression:

- Cyclin D-Cdk4 and Cdk6 complexes, along with Cyclin E-Cdk2 complexes, sequentially phosphorylate the retinoblastoma protein (RB) to facilitate the G1/S transition.

- Cyclin A-Cdk2 and Cdk1 are necessary for orderly S phase progression.

- Cyclin B-Cdk1 complexes control the G2/M transition and participate in mitotic progression.

b. 'Transcriptional' Cdks, which contribute to mRNA synthesis and processing:

- This group includes Cyclin H-Cdk7 and Cyclin T-Cdk9 (pTEFb), which phosphorylate the carboxy-terminal domain of RNA polymerase II to promote mRNA transcription elongation.

- Cyclin T-Cdk9 also regulates mRNA processing(20).

Cdk’s and cancer

Cyclin-dependent kinases (Cdks) and cyclins play critical roles in regulating cell cycle progression and transcription. Dysregulation of these biochemicals, including amplification, mutation, deletion, and hypermethylation of cyclins and their Cdk partners, disrupts cell cycle checkpoints and apoptotic activity, leading to proliferative disorders such as cancer. This dysregulation is directly linked to the molecular pathology of cancer.

Cell cycle progression through the G1 phase is primarily regulated by cyclin D-Cdk4, cyclin D-Cdk6, and cyclin E-Cdk2, which act on the retinoblastoma protein (RB) through sequential phosphorylations by Cdks. Genetic and epigenetic alterations observed in human cancers, such as mutations and amplification of Cdks and positive regulatory cyclin subunits, result in hyperactivation of Cdk regulatory pathways. Consequently, alterations in cell cycle checkpoints drive abnormal cell proliferation and tumor progression. While mutations in cdk genes within tumor cells are relatively rare, exceptions like Cdk4 and Cdk6 amplification occur. Overexpression or hyperactivation of fundamental cell cycle regulators is a common feature in various human tumors, including leukemias and carcinomas, and is associated with poor prognosis(19).

Inhibitors of apoptosis (Bcl-2)

Apoptosis, also known as "programmed cell death," is a natural process wherein cells undergo a series of events leading to their death once their function is fulfilled. Any deviations in the mechanism of apoptosis not only contribute to abnormal cell proliferation but also enhance resistance to anticancer therapies, such as radiation and cytotoxic agents. One of the suggested mechanisms for developing resistance to cytotoxic antineoplastic drugs involves alterations in the expression of B-cell lymphoma-2 (Bcl-2) family members.

The Bcl-2 family of proteins, comprising 25 pro- and anti-apoptotic members, maintains a balance between newly forming cells and dying cells. When there is an imbalance in the ratio of pro- and anti-apoptotic proteins, resulting in the overexpression of anti-apoptotic Bcl-2 family members, apoptotic cell death can be inhibited. Targeting the anti-apoptotic Bcl-2 family of proteins can enhance apoptosis and thus overcome drug resistance to cancer chemotherapy.

There are two major pathways of apoptosis: the intrinsic and extrinsic cell-death pathways(20).

1. The intrinsic cell death pathway, also known as the mitochondrial apoptotic pathway, is primarily triggered by internal stimuli. It is activated by various signals, including radiation, cytotoxic drugs, cellular stress, DNA damage, and growth factor withdrawal. This pathway involves the release of cytochrome c from the mitochondrial membrane space, which activates pro-caspase-9 and initiates apoptosis.

2. The extrinsic cell-death pathway functions independently of mitochondria and involves the cascade activation of caspases. Activation of cell-surface death receptors, such as Fas and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptors, directly activates the caspase cascade via an "initiator" caspase (caspase-8). The role of caspase-8 is to cleave other pro-caspases into active "executioner" caspases, which induce degradation of the cytoskeleton and nucleus.

Role of Bcl-2 in oral carcinogenesis

The Bcl-2 family comprises three distinct subfamilies distinguished by their structural and functional characteristics:

1. The anti-apoptotic subfamily, which includes Bcl-2, Bcl-XL, Bcl-w, Mcl-1, Bfl1/A-1, and Bcl-B proteins, contains all four Bcl-2 homology domains and acts to suppress apoptosis.

2. Some pro-apoptotic proteins, such as Bax, Bak, and Bok, are classified as multidomain proteins because they also contain Bcl-2 homology domains.

3. BH3-only proteins, such as Bim, Bad, and Bid, contain only the BH3 domain and function as pro-apoptotic proteins(21).

Recent studies have revealed the upregulation of Bcl-2 expression in oral squamous cell carcinoma (SCC). Bcl-2 inhibits cell death by suppressing apoptosis, suggesting that Bcl-2-mediated inhibition of apoptosis plays a significant role in the pathogenesis of oral SCC. Bax forms heterodimers with Bcl-2, and when present in excess, Bax overrides the anti-apoptotic activity of Bcl-2.

The p53 tumour-suppressor protein directly activates the human Bax gene transcription, implying that p53 may induce apoptosis through Bax-mediated suppression of Bcl-2 activity. Mutagenesis experiments have shown that single amino acid substitutions in Bcl-2 homology domains disrupt Bcl-2-Bax heterodimers, rendering Bcl-2 unable to inhibit apoptosis. However, the exact relationship between Bcl-2 and Bax remains controversial. Some studies suggest that Bcl-2 inhibits the apoptotic activity of Bax, while others propose that Bcl-2 and Bax heterodimers inhibit both apoptotic and anti-apoptotic activities, particularly in the presence of functional excess of Bax or Bcl-2, respectively.

Bcl-x and Bcl-2 form heterodimers with Bad, displacing Bax from Bcl-x and Bcl-2, thus enhancing apoptosis. Consequently, the Bcl-2 family, like the Myc family, operates partly through protein-protein interactions.In summary, Bcl-2-mediated inhibition of apoptosis is a significant factor in the pathogenesis of oral SCC. Moreover, by impeding apoptosis, Bcl-2 contributes to tumor cell resistance against anti-neoplastic drugs.

Tumor suppressor genes

Genes are responsible for encoding proteins that regulate negative signal transduction pathways and counteract excitatory pathways, thus maintaining a balance, and are commonly referred to as tumor suppressor genes(22). These genes play a crucial role in allowing cells to function effectively despite internal and external stresses.

It is recognized that oncogenes alone are insufficient to initiate oral cancer; rather, they serve as initiators in the process. The progression from a premalignant cell to a malignant one is primarily attributed to the inactivation of tumor suppressor genes, representing a significant event in malignancy development.

Various mechanisms contribute to the inactivation of tumor suppressor genes, including point mutations, deletions, hypermethylation, and rearrangements in gene copies. The identification of many tumor suppressor genes initially occurred in pediatric tumors, where inherited mutations in a single tumor suppressor gene were observed, leading to the formulation of the "Knudson two-hit hypothesis." This hypothesis proposes a genetic model for retinoblastoma development, with the inherited RB gene mutation being the first hit and the tumour-specific mutation acting as the second hit (23).

The mutant form of p53

Mutation of the p53 gene allows tumors to bypass the G1-S checkpoint, enabling the propagation of genetic alterations that may activate other oncogenes or deactivate tumor suppressor genes. Besides causing loss of function, mutations in TP53 can actively promote tumor development through various mechanisms:

1. Dominant negative manner: In situations where both wildtype (WT) and mutant alleles coexist (heterozygous state), mutant p53 can inhibit the tumor suppressor functions of WT p53 in a dominant negative (DN) manner. This interference occurs as mutant p53 disrupts the DNA binding activity of WT p53, which is essential for its transcriptional activity. However, this heterozygous state is often temporary, as TP53 mutations are frequently followed by loss of heterozygosity (LOH) during cancer progression, where the WT p53 allele is either deleted or mutated(22).

2. Gain of function: This term describes the acquisition of oncogenic properties by the mutant form of the p53 protein, beyond its simple inactivation. Throughout tumorigenesis, both dominant negative and gain of function effects may significantly contribute to the impact of missense mutations in the TP53 protein(23).

Mechanistic views of how mutant p53 exerts its function

Mutant p53 employs various mechanisms to contribute to tumor progression(22):

1. Gain of Function (GOF) Properties:

Mutant p53 acquires GOF properties that drive cells towards migration, invasion, and metastasis. Recent research reveals that mutant p53 can enhance cell migration and invasion. Notably, the oncogenic activity of Ras and the tumour-suppressor function of mutant p53 is not limited to early neoplasms; they also play crucial roles in later stages of tumor progression by facilitating TGFβ-induced metastasis.

2. Epithelial-to-Mesenchymal Transition (EMT):

Metastasis often involves the properties of epithelial-to-mesenchymal transition (EMT), characterized by the loss of cell-cell adhesion and increased cell motility. Mutant p53 has been identified as a promoter of EMT by facilitating the function of key transcriptional regulators in this process, such as TWIST1 and SLUG. In contrast, wild-type (WT) p53 has been shown to inhibit EMT mechanisms.

3. Tp63 Inhibition:

Another mechanism through which mutant p53 enhances cell invasion is by inhibiting the transcriptional activity of TAp63α. Notably, mutant p53 is unable to inhibit the function of TAp63γ, indicating a proto-oncogenic activity of TP53.

In certain cancers, p53 mutations are observed either late in the tumorigenesis process or play a significant role in advanced stages. However, some studies suggest its expression in early tumor progression stages. This discrepancy led to the hypothesis that TP53 mutations in early tumorigenesis lead to uncontrolled proliferation, a feature seen in both benign and malignant tumors, while mutations in later stages synergize with other oncogenic events to drive invasion and metastasis, characteristic of malignant tumors. The inactivation of p53 as a single event leads to a high proliferation rate. Inactivation of p53 combined with oncogenic H-Ras expression activates the expression of various chemokines and interleukins known to promote angiogenesis, invasion, and metastasis(24).

Tumor suppressor genes are generally believed to act recessively, requiring both gene copies to be inactivated for malignancy to occur. Loss of heterozygosity (LOH) and p53 mutations are reported in various tumors. There is controversy regarding the relationship between mutated p53 and its expression detected by immunohistochemistry. While some authors suggest a high correlation between p53 expression and point missense mutations, others report discrepancies, especially in oral cancer, attributing the lack of p53 expression in immunocytochemistry to insensitive detection methods for p53 mutation.

In Li-Fraumeni syndrome, mutant p53 exhibits instability similar to wild-type p53, suggesting that other events may be necessary for stability. The stability of mutant p53 is not intrinsic to its structure but may vary in different cellular backgrounds. This is exemplified by the relationship between p53 and Mdm2, where normal p53, when bound to Mdm2, is targeted for degradation via the ubiquitin-dependent pathway. However, mutant p53 fails to stimulate transcription of Mdm2, leading to its lack of degradation. Additionally, the E6 protein can form complexes with wild-type p53, promoting its degradation, which may explain the discrepancy between p53 mutation frequency and LOH. Other tumor suppressor genes include doc-1, the retinoblastoma gene, and APC.

Role of HPV in the pathogenesis of OSCC

The role of human papillomavirus (HPV) in the pathogenesis of human malignancies has been firmly established. HPV is a circular double-stranded DNA virus that primarily infects epithelial cells and is known to be the primary cause of cervical cancer, while its significance in oral carcinogenesis is currently being recognized. With over 100 subtypes, certain high-risk HPV subtypes are implicated in oral carcinogenesis, affecting approximately 85% of squamous cell carcinoma patients(25). Upon infection, the viral DNA becomes integrated into

the host genome, leading to malignant transformation. HPV carries two oncogenes, E6 and E7, whose open reading frames, E1 and E2, can be interrupted, resulting in overexpression of E6 and E7 proteins.

The E7 protein binds to the underphosphorylated form of retinoblastoma (pRb), leading to enhanced phosphorylation and degradation. The degraded pRb releases the E2F transcription factor, promoting cell proliferation by activating genes involved in cell cycle progression. Meanwhile, the E6 protein degrades the p53 protein, disrupting cell cycle regulation in infected cells, and marking the onset of HPV-mediated carcinogenesis. Due to the difficulty in culturing the virus, the role of HPV in oral squamous cell carcinoma (OSCC) pathogenesis is typically assessed by detecting viral DNA or expression of viral genes using PCR methods. Notably, the E6 and E7 proteins, known for their crucial role in cervical cancer, are also implicated in HPV-mediated carcinogenesis of the upper aerodigestive tract(26).

Immunotherapy

To evade detection by the immune system, tumor cells often increase the expression of inhibitory checkpoint receptors such as cytotoxic T lymphocyte antigen 4 (CTLA4), programmed death 1 (PD1), and its ligand PD-L1. This evasion strategy is particularly pronounced in head and neck squamous cell carcinomas (HNSCCs), where immune suppression is common, characterized by low levels of white blood cells and the presence of immune-suppressive tumour-infiltrating lymphocytes(27, 28).

Recent research has concentrated on the effectiveness of immunotherapies targeting these inhibitory checkpoint receptors in treating HNSCCs. Notably, inhibitors of PD-1 and PD-L1 have emerged as promising immunotherapies, promoting a durable anti-tumor response and stabilizing disease progression(29). Pembrolizumab and nivolumab, both anti-PD-1 receptor antibodies, have been approved for treating recurrent or metastatic HNSCCs that have not responded to platinum-based systemic agents. Combining nivolumab with ipilimumab, an antibody against CTLA4, has also shown success in treating refractory oral tongue SCC(30).

However, despite the progress with PD-1 and PD-L1 inhibitors, many cancers, including HNSCCs, continue to progress. Therefore, identifying predictive biomarkers is crucial for improving treatment outcomes. Several prognostic biomarkers, such as PD-L1 expression, tumor mutational burden, and immune gene signatures, have been extensively studied in HNSCC and other malignancies. These biomarkers help predict response to treatment with PD-1 and PD-L1 inhibitors, guiding personalized treatment approaches (31).

Tumor mutational burden, which reflects the total number of mutations per coding area of a tumor genome, has emerged as a prominent biomarker for the response to PD-1 inhibitors. Cancers with higher mutational burdens tend to respond better to PD-1 inhibitors, possibly due to the increased formation of tumour-specific antigens that the immune system can recognize (32).

Additionally, the activation status of immune cells in the tumor microenvironment serves as another biomarker for response to PD-1 and PD-L1 inhibitors. Studies have shown that the expression of specific immune genes correlates with improved response rates, progression-free survival, and overall survival in HNSCC patients treated with pembrolizumab. These findings underscore the importance of understanding the tumor immune microenvironment in

tailoring immunotherapy approaches for HNSCC patients (33).

Conclusions

Cellular signaling pathways are not discrete entities but rather intricately interconnected, forming complex signaling networks. Any alteration or diversification in these networks, such as elevated production of growth factors or cell surface receptors, increased levels of transcription or translation, or changes in intracellular messenger levels, can lead to abnormal cell proliferation. This phenomenon contributes to the multifactorial nature of oral carcinogenesis. Such changes have the potential to activate proto-oncogenes or suppress tumor suppressor activity, resulting in a cellular phenotype characterized by enhanced proliferation, weakened cell cohesion, and the propensity for local infiltration and metastasis.

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All authors reviewed the results and approved the final version of the manuscript

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