2024
11(1), 652

Chemical Biology
LETTERS

Signaling and molecular pathways implicated in oral cancer: A concise review

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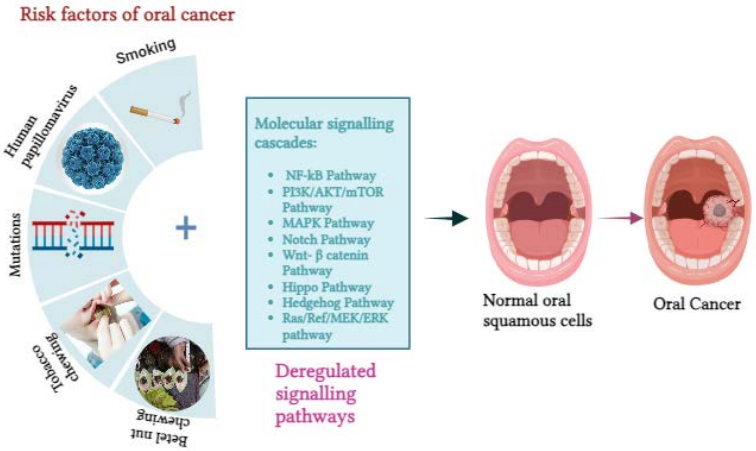
Submitted on: 04-Aug-2023, Accepted and Published on: 02-Nov-2023

Review

ABSTRACT



Oral cancer is the sixth most prevalent type of cancer worldwide and third in India out of the different cancer types identified. Mouth and oral cancers collectively refer to cancers of the buccal cavity, lips, oropharynx, hypopharynx, and larynx. Genetic anomalies, the upregulation of several proteins, the deregulation of tumor-suppressive and oncogenes, and risk factors like alcohol and tobacco consumption are a few examples of the known irregularities that contribute to the development of oral cancer through the accumulation of various carcinogenic substances. Oral cancer is caused and developed by multiple molecular and cellular pathways such as PI3K/AKT/mTOR, Ras-Raf-MEK-ERK pathway, Wnt signaling, NF-κB pathway, Hippo pathway, etc. In addition, various genes including TP53, PTEN, CDKN2A, HRAS, PIK3CA, NOTCH1, IRF6, TP63, etc. are also involved in this malignancy. Therefore, it is crucial to have a deep understanding of these pathways to properly understand the development of oral cancer. This short review focuses on compiling together various signaling and molecular pathways accountable for oral carcinoma development.

Keywords: Oral cancer, PI3K/AKT/mTOR signaling, prognostic biomarker, NF-kB pathway, dysregulation

INTRODUCTION

Cancer is one of the leading causes of mortality worldwide. According to a 2020 report by GLOBOCAN, there were about 10.3 million deaths due to cancer and around 19.3 million new annual cases of cancer globally.[1,2] Around 77,000 new cancer cases and 52,000 deaths due to cancer are recorded annually in India.[3] In India, the anticipated total cancer cases and the rate of incidence for the year 2022 was found to be 14,61,427 (100.4 per 100,000), with more female cases (7,49,251; 105.4 per 100,000) than male cases (95.6 per 100,000).[4] The fourth prevalent type of cancer among these was cancer of the oral cavity and pharynx (1,98,438 cases), with a higher incidence in males than in females.[4] Oral cancer or mouth cancer is any cancer that appears in the oral cavity, including the mouth. Oral cancer falls under the category of head and neck cancer. 95% of all cases of head and neck cancer (HNC) are caused by oral squamous cell carcinoma (OSCC), and in the last ten years, its frequency has increased by 50%.[5] The anatomical area between an imagined coronal plane drawn from the intersection point of the soft palate and the hard palate, the tongue's circumvallate papillae, and the lip vermillion is known as the oral cavity, and it mainly has seven sub-sites – hard palate, soft palate, lip, retromolar trigone, the floor of the mouth, alveolar, tongue.[6] The incidence and mortality brought on by oral cancer tumors vary depending on the region in which it is discovered.

Due to deferred identification and lack of effective early-detection biomarkers to detect the disease, the clinical prognosis of this cancer is typically poor. An early identification of the disease is crucial to lower the death rate of patients with oral

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URN:NBN:sciencein.cbl.2024.v11.652
DOI: 10.62110/*sciencein.cbl.2024.v11.652*

 ISSN: 2347–9825
https://thesciencein.org/cbl

cancer. A wide range of techniques for detecting oral cancer, *viz.* physical examination, spectroscopy (autofluorescence Raman, FTIR), DNA microarray, biopsy, imaging techniques (MRI, Ultrasound, CT scan, PET), histological staining, etc., as depicted in Figure 1 are in current use.[7]

Treatment strategies used for oral cancer in general are surgery, chemotherapy, radiotherapy, immunotherapy, etc. Apart from various genetic irregularities like DNA mutations, genetic changes, dysregulated signaling pathways, irregulated oncogenes, tumor suppressor genes, and altered levels for specific proteins, a few risk factors causing oral cancer comprise tobacco usage, especially smokeless tobacco, excessive alcohol use, chewing betel nut, deprived oral hygiene, and recurrent viral infections like the human papillomavirus[7], poor nutrient uptakes, age. Multiple genes playing significant roles in oral cancer incidence involve cell cycle regulating genes or oncoproteins (Ras, cyclins, Myc, CKIs, CDKs), pro-survival proteins (like telomerase, growth factors and/or receptors), pro-apoptotic proteins (Bax, Fas, caspases, BH-3 family, TNF-R), tumor suppressors (p53, pRb), anti-apoptotic proteins (Bcl2 family, NF-kB, IAPs), genes for transcription factors etc.[8] Since genetic factors play a crucial role in oral cancer development, therefore, a deeper understanding of molecular and signaling pathways involved in oral cancer is essential for therapeutics and diagnostics development. Several signaling pathways like PI3/AKT signaling, JAK/STAT signaling, Notch signaling, Wnt β-catenin pathway, mTOR signaling, hedgehog pathway, MAP Kinase pathway[9], Ras/Raf/MEK/ERK pathway, etc. are involved in causing various types of cancers. This review discusses the different molecular and cellular pathways involved in oral cancer development.

PATHWAYS INVOLVED IN ORAL CANCER

1. PI3K/AKT/MTOR SIGNALING

Among the most vital intracellular mechanisms governing cellular growth, cell motility, cellular survival, cellular metabolism, and angiogenesis is the PI3K/AKT/mTOR signaling system.[10] Numerous cancers' development and progression are linked to the activation of AKT. Typically, the PI3K/AKT/mTOR axis controls cell metabolism, growth, and result in the malignant phenotype in various cancers including HNC.[11] MAP kinases and PI3K/AKT/mTOR signaling pathway are thought to encourage uncontrolled cell proliferation by inhibiting PTEN, which boosts cell survival and speeds up cancer development.[12] PI3Ks are a multimember family of three types of heterodimeric lipid kinases. Receptor tyrosine kinases (RTKs), the catalytic subunit (p110), and the p85-like regulatory subunits, trigger Class IA PI3Ks, as shown in Figure 2.[13] G protein-coupled

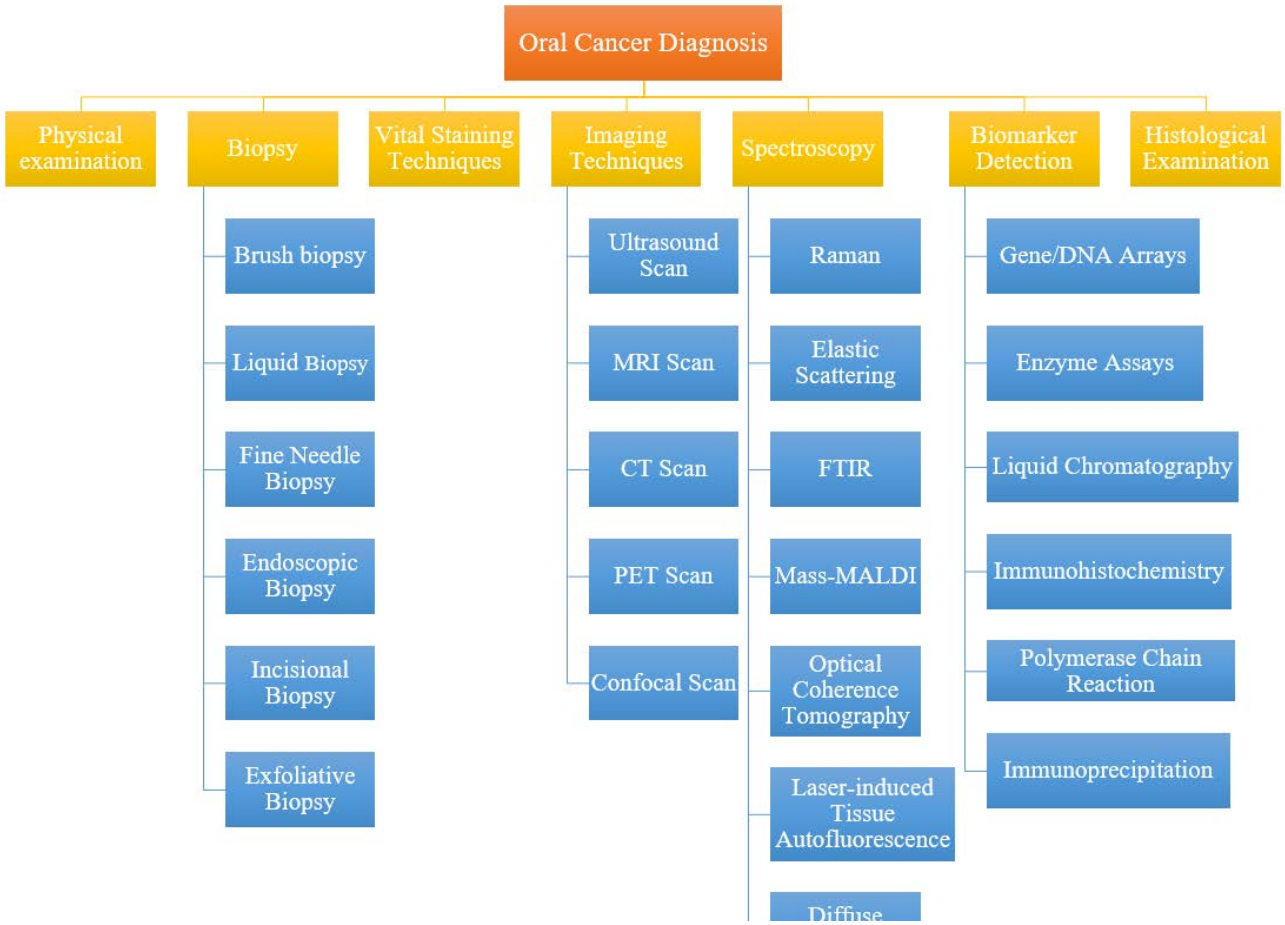

Figure 1: Oral cancer detection techniques. Image idea taken from Borse et.al [6]

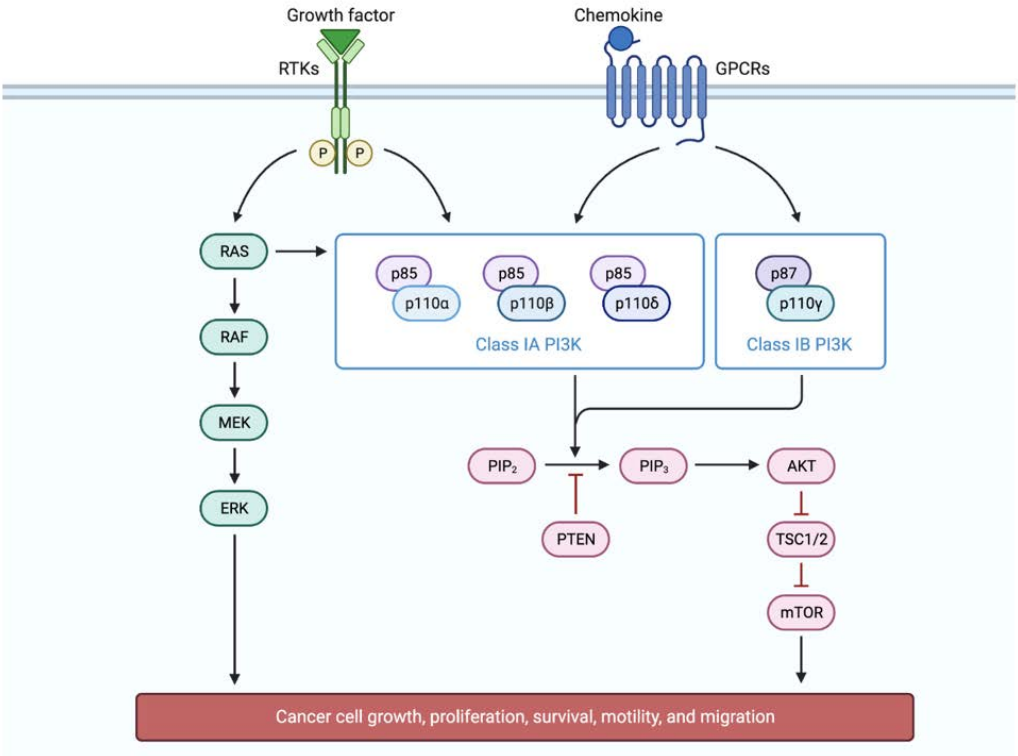

Figure 2: Role of PI3K signaling cascade in cancers. RTKs on phosphorylation activate different types of PIKs, which in turn activate AKT-TSC1/2-mTOR cascade affecting the growth and proliferation survival of cancerous cells. (source: Biorender)

survival in healthy physiology. However, changes in this system receptors (GPCRs) and the regulatory subunits activate Class IB PI3Ks. PIK3C2A, PIK3C2B, and PIK3C2G are the three proteins comprising Class II PI3Ks. The Class III PI3Ks have just one single member, PIK3C3. PI3K is linked to the serine/threonine kinases (AKT and PDK-1), which have PIP3-binding Pleckstrin Homology (PH) domains in several cells.[13,14] AKT is a serine protein kinase that has been conserved during evolution and possesses three parts in its structure - 1. *N*-terminal Pleckstrin homology domain, 2. C-terminal tail with a regulatory motif of hydrophobic nature, 3. Linker section, which has a central kinase having a part of catalytic nature.[15] AKT gets recruited to the cellular membrane by its PH domain when there is an increase in the levels of PI(3,4,5)P3, by an accumulation of PI(3,4)P2 (reduced extent), and performs its catalytic functions by triggering threonine phosphorylation induced by PDK1 and serine phosphorylation mediated by mTORC2.[16,17] Multiple downstream targets of activated AKT are involved in cell migration, angiogenesis, growth, and proliferation.[18] One of the most important downstream targets of AKT is another serine/threonine protein kinase called mTOR and mTOR complex1 (mTORC1), which is its catalytic subunit. mTORC1 phosphorylates the components of protein synthesis and regulates protein translation.[11] PTEN, a mTOR inhibitor, promotes autophagy, whereas Ras, PI3K, and AKT oncogenes stimulate mTOR and thus reduce autophagy.[19] Targeted medicines against this pathway have been demonstrated to be quite effective in plummeting the tumor burden in animal models as well as in clinical settings. Intending to study the role of PI3K/AKT signaling pathway in oral carcinomas Xia *et al.* conducted a study on oral cancer squamous cell lines U1 and SCC25 using oridonin, a natural diterpenoid and found that oridonin inhibited the colony formation, cancer cell proliferation, and induced apoptosis and the G2/M cell cycle arrest (by downregulating cyclin B1, upregulating cyclins D1, D3, p21, p-CDK1, and cyclin A2) in two cell lines.[20] The anti-cancerous action of oridonin was also shown due to the inhibition of phosphorylation of PI3K/AKT thus, affecting the PI3K/AKT signaling pathways, suggesting this pathway's role in oral cancer.[20] Another validation of the role of PI3K/AKT signaling in oral cancer can be deduced from the study conducted by Das *et al.*, where the group studied the anti-cancer effects of a few PI3K inhibitors, namely PI-103, PI-828, and PX-866 on SCC-4, SCC-9, and SCC-25 cell lines.[21] The study concluded that targeting the PI3K/AKT pathway by PI3K/AKT inhibitors showed anti-cancer activities by inhibiting inflammation (COX2 downregulation), angiogenesis (VEGF downregulation), arresting cell cycle (S and G2/M phase arrest), enhancing apoptosis in all the three cell lines.[21] The contributed role of PI3K/AKT/mTOR signaling cascade with its effect on multiple factors plays a vital role in the incidence and progression of oral cancer, as evident from various studies.

2. Nuclear Factor kappa B (NF-kB) pathway

NF-kB signaling pathway majorly plays a role in the survival of cells, initiating the immune response, inflammation, cancer progression, chemoresistance, etc. NF-kB is a transcription factor usually reserved in the cell cytoplasm by its inhibitory protein (IkB), whose phosphorylation leads to its ubiquitination, thus releasing NF-kB, which is then free to enter the cell nucleus and regulating various gene expressions as depicted in Figure 3 with a role to play in apoptosis, cell survival, cell proliferation.[22] NF-kB controls the expression of target genes like Bcl2, TNFA, VEGF, Bclxl, IL6, Bclxs, XIAP, etc., and mediates cell proliferation, angiogenesis, and survival in cancer cells.[23] NF-kB consists of five subunits-Rel, p65, RelB, p105/p50 and

p100/p52.[24 25] These subunits can form homologous and heterologous dimers and bind to specific sequences (NFkB sites) of target genes to regulate their gene transcription. Through the tiny variations in how these NF-kB dimers attach to specific sequences, NF-kB controls the activity of cells.[26] Increased NF-kB levels cause a rise in Interleukin-6, which boosts the signal transducer and transcriptor activator levels.[27] NF-kB targets the enzyme cycloxygenase-2 (COX-2), which produces prostaglandins from arachidonic acid.

Cancer spreads through overexpressing COX-2 and encouraging cellular proliferation, vascularity, and evading the process of apoptosis.[22] A study by Anbo *et al.* reported that the knockdown of the NF-kB/p65 subunit caused growth inhibition in oral cancer cells under hypoxic conditions.[28] Another study investigated the role of curcumin, a natural antioxidant, on HPV16 positive oral cancer cell line and found that curcumin downregulated the expressions of the transcription factors NF-kB and AP-1 by changing the NF-kB composition from p50/p50 to p50/p65. Conclusively, it downregulates the

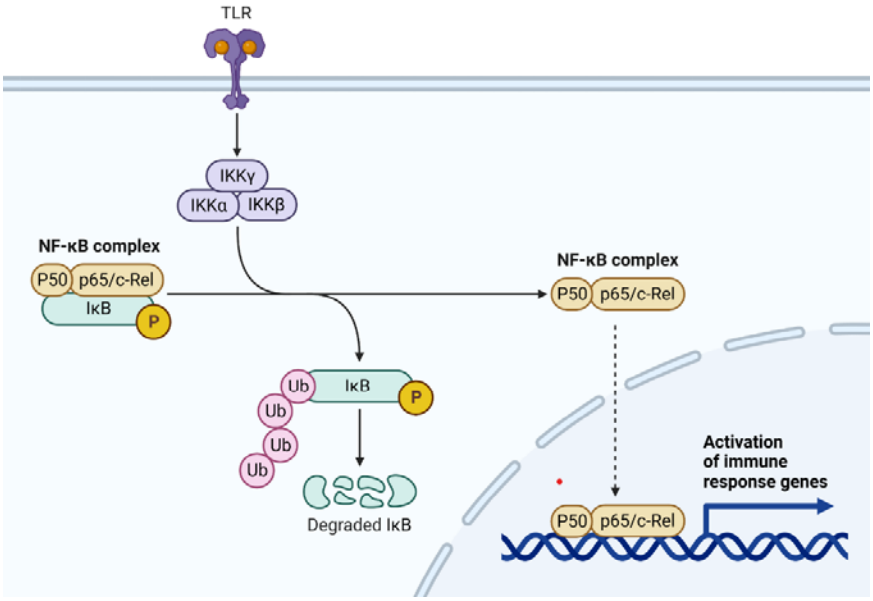

Figure 3: Activation of NF-kB complex by degradation of IκB post phosphorylation. (source: Biorender)

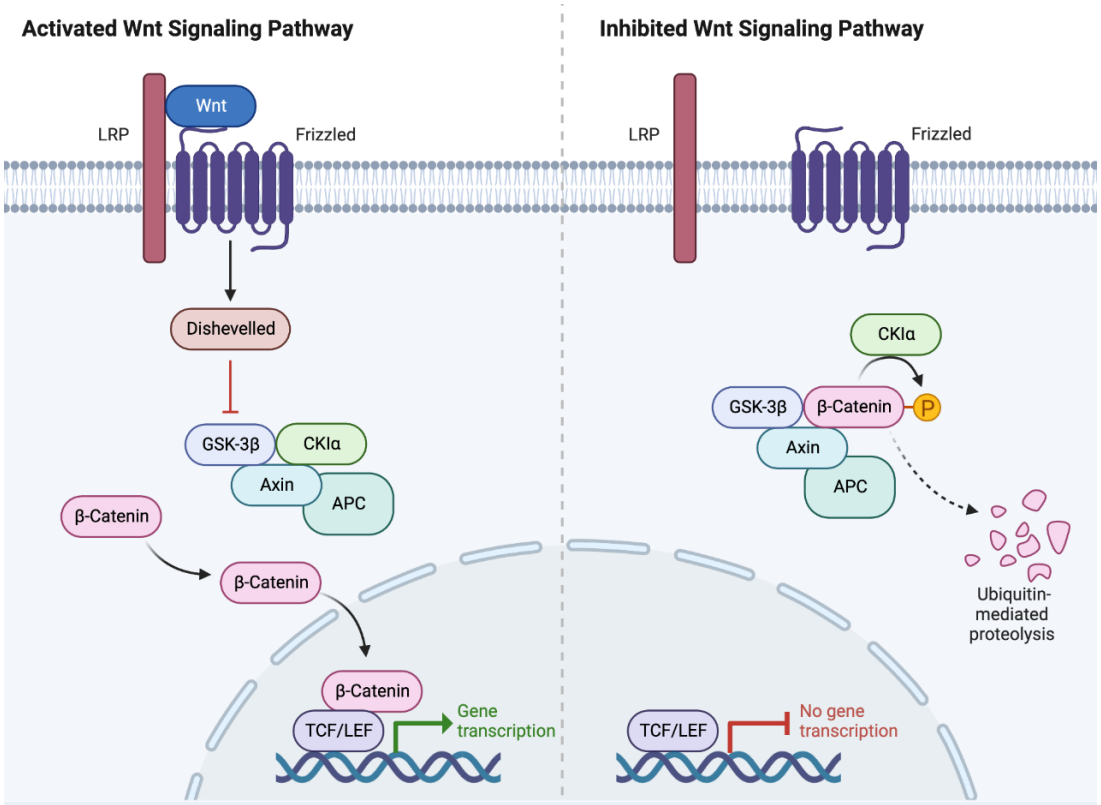

Figure 4: Wnt/β- catenin pathway gets activated when Wnt binds to its frizzled and LRP5/6 receptors activating the dishevelled protein which suppresses the GSK-3β that helps in the internalization of the β- catenin to bind to TCF/LEF transcription factors which in turn activate the target gene transcription. In the absence of the Wnt ligand GSK-3β ternary complex of AXIN, Axin, APC, CK1α forms a complex with β- catenin which causes the ubiquitin-mediated proteasomal degradation of this complex rendering the pathway inactivation (source: Biorender).

HPV16 E6 protein and decreases oral cancer cell viability, thus suggesting a significant role of NF-kB in oral cancer.[29] Another investigation into the clinical significance of NF-kB and TGF- β1 was conducted by Lilan *et al.*[30] The group found that primary OSCC and metastatic lymph nodes had considerably higher expression of NF-κB, while the expression levels of TGF- β RII were higher in the surrounding normal tissues and inflammatory lymph nodes. NF-kB can be of use as an independent prognostic factor and is related to OSCC patient prognosis as it is related to the degree of lymph node metastasis OSCC differentiation.[30]

3. WNT SIGNALING PATHWAY

The Wnt pathway plays a pivotal role in various types of cancers like breast, lung, and HNC, and in the case of oral carcinoma, the dysregulation of this particular signaling pathway is allied to the prognosis of OSCC patients. For activation of the Wnt signaling cascade, the Wnt ligand needs to bind to the receptor to trigger multiple intracellular events thus initiating an intracellular signaling cascade resulting in gene transcription as depicted in Figure 4. This is demonstrated in the case of Wnt7b, a Wnt/β-catenin activator that is significantly expressed in OSCC patient specimens as compared to matched nearby non-tumorous tissues.[31] Comparing patients with high tumor Wnt7b expression to those with low tumor Wnt7b expression, patients with high Wnt7b expression often had considerably lower disease-specific survival rates.[32] Wnt pathways are mainly of two types- canonical or β-catenin dependent and non-canonical or β-catenin independent[33] and play a role in various cancer cell mechanisms like cell proliferation, cell survival, polarity and migration of cancer cells, determination of cell fate, self-renewal in stem cells.[34] Various Frizzled and Wnt genes like Wnt5a and Fzd5 are expressed in oral cancer, and Wnt5a plays a role in stimulating non-canonical Wnt/Ca2+/PKC pathway, oral cancer cell invasion, and migration, suggesting increased Wnt5a levels in tumorous tissues induce oral carcinogenesis and can be used as a biomarker for oral cancer detection as well.[35-37] A study steered by Kleszcz *et al.* for the identification of appropriate molecular targets of Wnt signaling down-regulation in HNC cells found that inhibiting the porcupine and CBP/β-catenin interaction by PRI-724 and IWP-2, respectively had a strong effect on β-catenin dependent gene expression in head and neck squamous cell carcinomas by apoptosis induction and cell migration. [38] Further studies conducted by Kleszcz *et al.* for evaluation of combinatorial effects of glycolysis and Wnt inhibitors on tongue cancer cells (CAL 27, SCC-25) reported that this combination of Wnt signaling and glycolysis inhibitors attenuates tongue cancer cells by reducing the cellular viability, apoptosis induction, G0/G1 cell cycle arrest.[39] These studies suggest the role of the Wnt signaling pathway in oral cancer.

4. NOTCH SIGNALING PATHWAY

Notch signaling is a vastly conserved signaling pathway that generally occurs between cells requiring cell-to-cell contact and is present in human tissues ubiquitously. The two primary functions Notch signaling performs include determining cell fate asymmetrical division, which is essential for proper embryo growth. Anomalous Notch signaling influences the pace of cell proliferation, the tumor microenvironment, and stem cell activity, which contributes to the advancement of cancer. Also, Notch signaling plays a dependent role in various cancers. For example, it is highly active in breast cancer and T cell lymphomas, but in the case of skin and oral cancer (SCC), its expression is suppressed.[40] There are four types of receptors: Notch 1,2,3, and 4, and two types of ligands- Jagged (Jag 1 and 2) and Delta-like ligands (Dll 1,3,4). Notch signaling can be either canonical, which depends on CBF1/RBPjk/Su(H)/Lag1 (CSL) transcription factors, or non-canonical, which works independently of the CSL complex. This is evident in a study conducted by Zhang *et al.* Jag1 expression clampdown inhibited cell proliferation in tongue squamous cell carcinoma (TSCC), implying its essential role in interacting with different Notch signaling molecules.[41] In the case of tongue carcinoma, the mRNA levels of Notch 1, Notch 3, Jagged 1, and Jagged 2 are upregulated compared to the adjacent no-tumorigenic tongue tissues.[42] A study conducted by Zhang *et al.* to investigate whether notch signaling plays any role in epithelial-mesenchymal transition (EMT) and metastasis in OSCC reported that Snail protein regulated the EMT and promoted migration of OSCC tumor.[43] Also, to verify the role of the Notch pathway in EMT, the pathway was inhibited with DAPT, a type of γ-secretase inhibitor which caused the decrease

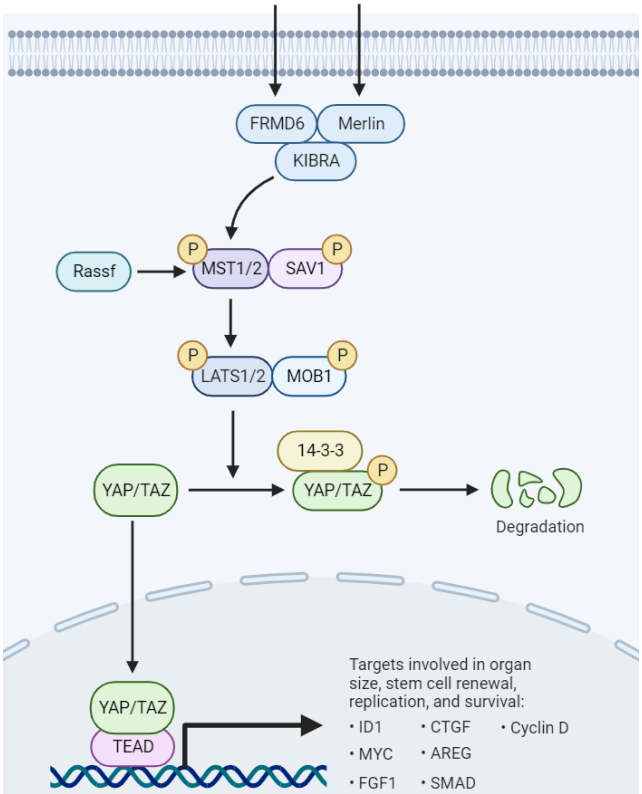

Figure: 5 Phosphorylated MOB in the hippo pathway recruits transcription factors (TFs) like the SAV, MST1/2, which, on their phosphorylation further phosphorylate YAP/TAZ, causing its translocation to the nucleus and form a dimer with TEAD. This leads to activation and transcription of other TFs, leading to cancer development (source: Biorender).

in the migration potential of the OSCC cells, suggesting that the Notch pathway plays a role in EMT and metastasis.[43] A Similar kind of study under hypoxic conditions by Ishida *et al.* also reported upregulated ligands of the Notch pathway-Notch 1-4, both Jag1, Jag2, and DLL4, and showed increased cell motility and invasion.[44] The expression of two Notch target genes, HEY1 and HES1, was also amplified, causing enhanced cell proliferation under a hypoxic environment, thus establishing the relation between hypoxia and the Notch pathway in oral cancer.[44] High levels of NOTCH1 and Jagged1 are generally required for the stemness ability of stem cells in cancers and to enhance cell-to-cell communications. The Notch signaling component's interactions with molecules from other signaling pathways impact oral cancer growth in various ways and vice versa.

5. Mitogen-activated protein kinase (MAPK)/ERK signaling pathway

MAPK pathway is generally involved in regulating various proteins that play a role in apoptosis, tumor cell proliferation, angiogenesis, differentiation, metastasis, and invasion. MAPK signaling pathway has four sub-pathways – the c-Jun N-terminal kinase (JNK) pathway, extracellular signal-regulated kinase (ERK-1/2) pathway, ERK5 pathway, and p-38 pathway.[45] These pathways correlate with different MAPK kinase families like JNK1/2/3, ERK1/2, ERK5, and p38α/β/γ/δ, respectively, which are the protein chains that control expressions of various genes by transducing other extracellular signals to the nucleus.[46] The multiple components of these four sub-pathways include Ras, Raf, MEK, MAPKK, TNF- α, IL-β, ATF-2, ELKI, ETS, etc., which by modulating different factors affect the cellular process like promoting tumor cell proliferation, inhibiting apoptosis, promoting tumor angiogenesis, inducing tumor invasion and metastasis in oral cancer.[47] In a study conducted by Dey *et al.* to explore the role of calcium-binding protein, S100A7 in oral cancer, it was revealed that its activation modulated the MAPK signaling pathway by RAB2A pathway and p38 mitogen-activated protein kinase suggesting its role in oral carcinomas.[48] S100A7, also an early OSCC detection marker when knocked down by RNA interference, showed inhibited cell proliferation, growth, migration, and invasion and induced apoptosis in the in-vitro culture conditions.[48] Another evidence of the role of MAPK/ERK in oral cancer can be deduced from the study conducted by Zhang *et al.*, where the involvement of MAPK/ERK signaling in oral carcinoma was explored by investigating the effect of miR-214 and revealed that it increased p-ERK levels, p-ERK/ERK levels thereby activating MAPK/ERK signaling causing raised PCNA levels, increased levels of p21 which brought about the inhibition of apoptosis, promoted proliferation of oral cancer cells.[49] MAPK signaling pathway also plays a role in the metastasis and invasion in oral cancers, as revealed by the study conducted by Zhao *et al.*, where the impact of MAPK pathway in EMT was investigated in OSCC and was found that epithelial to mesenchymal transition was promoted by TNF-α which was upregulated by components of MAPK signaling sub-pathways *viz.* JNK, p38, ERK.[50]

6. Hippo signaling pathway

Among various molecular signaling pathways pivotal in regular and cancerous cell development, the hippo signaling pathway is dysregulated in multiple cancers like breast, colorectal, liver, head and neck, and oral cancers. It generally regulates organ size, tissue growth, and stem cell attributes by regulating cell-to-cell contacts, cellular apoptosis, and cell proliferation, and its dysregulation plays a role in tumorigenesis. Hippo signaling cascade consists of four core components – the large tumor suppressor (LATS) kinases, Mps One Binder kinase activator protein (MOB1), mammalian sterile 20-like (MST) kinases, adaptor proteins Salvador homolog1 (SAV1), which inhibit processes contributing to tumorigenesis by activating the downstream transcriptional cofactors, Yes-associated protein 1 (YAP1) and transcriptional coactivator with PDZ-binding motif (TAZ) as shown in Figure 5. [51] Hippo pathway functions via two different methods, canonical and non-canonical with their various upstream regulators like RASSF, NF2/Merlin, KIBRA, FRMD6, GPCR, FAT4, CD44, AMPK, SCRIB for canonical pathway while PTPN14, AMOT, ZO-1/2, β-Catenin/α-Catenin, YES1, c-ABL, AMPK, VGLL4 for non-canonical pathway.[49] Many studies to explore the role of hippo signaling in oral cancer have been performed, and the role of various components has been elucidated. For example, Li *et al.* showed that the downstream effector of hippo signaling, TAZ, promoted cell proliferation, chemoresistance, migration, and invasion, induced EMT, TGF- β1- induced EMT in OSCC.[52]

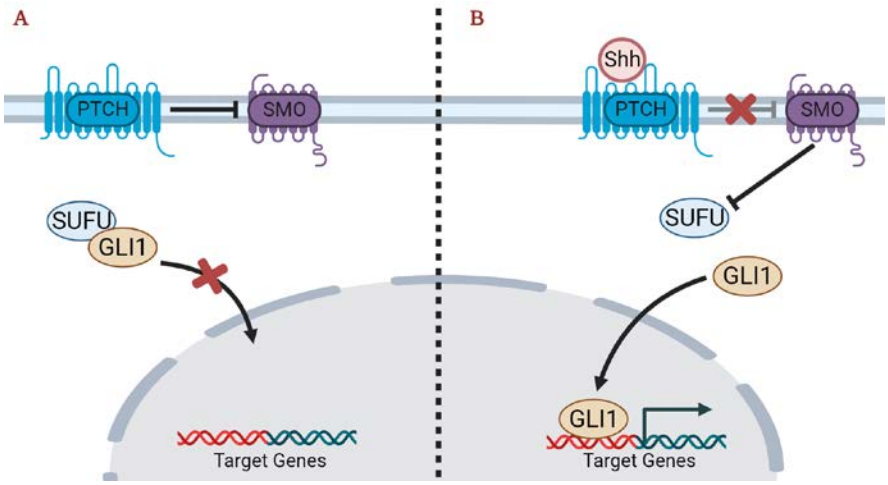

Figure 6: A) Hedgehog signaling remains inactive when the ligand is absent, thus inhibiting the Smo activity and preventing localization of Gli to the nucleus, further repressing the target genes of hedgehog pathways like the cyclins E, cyclin D, and myc, which play an essential role in cell proliferation regulation and angiogenesis. B) The target genes are successfully activated when the ligands (SHH, IHH, DHH) are present (source: Biorender).

Another study by Wei *et al.* in tongue squamous cell carcinoma (TSCC) provided clinicopathological evidence of anomalous overexpression of TAZ in poor survival of TSCC and Ki-67 abundance.[53] The TAZ protein and mRNA levels were heightened in TSCC compared to the normal tongue mucosa, showing TAZ's critical role in tumorigenesis.[53] Various miRNAs and lncRNAs also have roles to play in different types of cancers by the impact on various components of different signaling pathways. One such example is lncRNA LEF1-AS1, which was upregulated in OSCC, and its knockdown inhibited cellular proliferation and survival of the carcinomas by affecting the phosphorylation process of core components, MST1/2, LATS1/2, SAV1, YAP and MOB of the hippo signaling pathway thus rendering hippo cascade inactive. LEF1-AS1 influenced YAP1 cellular distribution via LATS1-mediated mechanisms, confirming that LEF1-AS1 inhibited hippo signaling activity in oral squamous cell carcinoma.[54]

7. HEDGEHOG SIGNALING PATHWAY

Hedgehog (HH) signaling is the most critical pathway of early embryonic development or embryogenesis and performs roles in a concentration-dependent fashion in tissue patterning, cellular growth, vascularization, differentiation, adult stem cell population maintenance, and cell proliferation.[55,56] The core components of the HH pathway include three ligands known as Sonic Hedgehog (SHH), Indian hedgehog (IHH) and Desert hedgehog (DHH), and three glioma-associated oncogene homolog (GLI) transcription factors- GLI1, GL2, GL3, patched receptors (Ptch1, Ptch2), smoothened receptor (smo), kinesin protein Kif7, suppressor of fused homolog (Sufu), cyclic adenosine monophosphate (cAMP), protein kinase A (PKA)[57,58] Hedgehog (HH) ligand, Patched (Ptch), and Smoothened (Smo) proteins are involved in HH signaling activation as depicted in Figure 6. HH signaling pathway activation occurs in different ways in different cancers- GLI dependent-classic activation by ligand binding (canonical) and non-classical activation in the absence of ligand (non-canonical), activation of the non-canonical pathway by other signaling pathways like PI3K, RAS, and due to mutations causing overproduction of ligands leading to constitutive activation of HH cascade in cancers like breast cancer, gastric cancer etc.[59] Various studies in oral cancer have revealed that HH has significant roles in the tumor microenvironment in OSCC.[60] SHH acts as a predictive biomarker [61] and has increased the expression of SHH protein in OSCC.[62] An investigation conducted by Cierpikowski *et al.* to study the importance of SHH expression in OSCC showed it was activated and acted as an independent prognostic factor contributing to poor overall survival of OSCC patients.[61]

ASSOCIATION BETWEEN SMOKING, TOBACCO CHEWING, AND ORAL CANCER

Smokeless tobacco, viz. chewing betelnut, snuff, dip, and smoking is responsible for increased risks of oral cancer development. Smokers are 5-10 times more likely to develop OC than nonsmokers, and the risk rises with increased exposure frequency.[63,64] An investigation conducted by Gupta *et al.* to study the impact of tobacco smoking on oral cancer genetics using next-generation sequencing (NGS) identified 31 genes at 28 different genetic loci altered in tobacco smoking patients.[65] p53 signaling pathway is another pathway that showed mutations in p53 with higher frequency in smokers than in nonsmokers and played roles in local recurrence, effect on the clinicopathological features and survival in OC patients.[66] Barui *et al.* have reported that cigarette smoking altered the mRNA levels of different genes and transcription factors like MAPK1, β- catenin, TNF- α, PARD 3, FZD1, and vimentin.[67] The group concluded that the development of OC under the influence of smoking cigarettes progresses due to multiple effects, including canonical Wnt/MAPK pathway activation.[67] miRNA dysregulation due to cigarette smoking can also accelerate OC progression, as demonstrated by a study by Shieh *et al.*[68] As per their investigations, the expression levels of the miR-30amRNA were downregulated on treatment with the cigarette smoke condensate (CSC) in YD38 and SCC25 cells. CSC treatment showed enhanced expression of VEGF in OSCC cells, a downstream regulator of the binding immunoglobin protein (BiP). BiP is critical in EMT, migration, invasion, and tumor-associated angiogenesis. The group's observation concluded that miR-30a downregulation by CSC and, as a result of miR-30a downregulation, the epigenetic regulation of BiP plays a role in the CSC-induced progression of the OSCC.[68] Another study by Chanh *et al.* reported that areca nut or betel nut caused increased levels of ROS in SAS, OC3, and OECM-1 OSCC cell lines by modulating NF-κB, which increased ROS-activated p38 and MKP-1.[69] Areca nut induced autophagy in the OSCC cells by upregulating the HIF-α levels while inactivating ERK.[69]

COMPOUNDS IN CLINICAL TRIALS FOR ORAL CANCER TREATMENT

Chemotherapeutic medications are a crucial component of the oral cancer treatment plan. They are employed to either eliminate or inhibit the proliferation of cancer cells. Depending on the stage and kind of oral cancer, several chemotherapy medicines and medication combinations may be utilized for treatment. Various compounds and drugs have been investigated as potential drug candidates to inhibit different molecular pathways or pathway components and are in clinical trials. In a study by Zhong *et al.* to explore neoadjuvant combinations of camrelizumab and apatinib, it was reported that this combination is safe to treat locally advanced OSCC (NCT T04393506).[70] A study conducted by Xia *et al.* revealed that diterpenoid oridonin has anti-oral cancer potential as it induced apoptosis, G2/M- phase cell cycle arrest, and inhibited the PI3/Akt signaling pathway in oral cancer cell lines UM1 and SCC25.[20] Many clinical trial studies are being conducted to look for compounds effective in controlling oral cancer or the management of premalignant lesions in oral cancer, like metformin hydrochloride with extended-release QDS for preventing oral cancer in patients with oral premalignant lesions (NCT02581137)[71], rosiglitazone maleate for treating oral leukoplakia patients (NCT00369174), pioglitazone hydrochloride for preventing HNC in oral leukoplakia patients (NCT00099021), nivolumab for treating the oral proliferative

verrucous leukoplakia (NCT03603223), sintlimab to prevent cancerization of premalignant lesions (NCT04065737), vandetanib to prevent HNC in patients having (NCT01414426), sodium valproate for managing high risk oral epithelial dysplasia (SAVER)[72], avelumab as immune checkpoint inhibitor in high risk oral premalignant lesions (NCT04504552).[73]

Discussion

The involvement of multiple molecular and cellular pathways in the development and progression of oral carcinomas makes it necessary for a critical and deeper understanding of the underlying pathways for the efficient management of oral cancer. PI3K/AKT/mTOR signaling cascade and RAS/RAF/MER/ERK pathway play crucial roles in oral cancer tumorigenesis as most of the components of these pathways are generally overexpressed or activated in oral cancers. NF-κB signaling cascade regulates various genes like VEGF (role in tumor angiogenesis), Bclxl, and other immune system genes, which play a significant role in cancer progression and development. MAPK signaling pathways play essential roles in apoptosis, tumor cell proliferation, metastasis, etc., and can act as a target pathway to control tumor metastasis. The Wnt signaling pathway plays a role in stem cell renewal, differentiation, and proliferation of the tumor cells. It can act as a potential target pathway to impede the immortality of the tumor cells. The Notch signaling system is a juxtracrine signaling route that activates the genes linked to cell survival, angiogenesis, and proliferation, and its components are usually overexpressed; hence its suppression can be a way to inhibit oral cancer. Hedgehog signaling involves a wide range of biological processes, including the start of cellular growth and division, lineage specification, axon guidance, and survival-related tasks. Mutations and aberrant expression of this pathway cause carcinogenesis and impact oral cancer patients' overall survival. The Hippo signaling system is essential for the development of tumors, control of stem cell homeostasis, tissue regeneration, and regulation of organ size. Overall, the above-discussed signaling pathways play significant roles in oral cancer development, progression, and diagnosis and can be used as therapeutic targets to manage oral cancer.

Conclusion and Future Perspectives

Oral cancer, with a relatively high incidence rate worldwide, has become quite a nuisance in mainly developing countries owing to lifestyle adaptations viz consuming alcohol, smoking, tobacco chewing, etc. This unhealthy lifestyle leads to mutations in various oncogenes tumor suppressor genes and dysregulates different molecular cascades and signaling pathways either by upregulating or downregulating a particular component or the complete path as such and causing the initiation or progression of oral carcinogenesis. The most frequent type of head and neck cancer is oral cancer, which is caused by disrupting one or more signaling pathways and is involved in various other types of cancers like PI3K/AKT/mTOR, Wnt-β, hedgehog, MAPK, Hippo, JAK/STAT, Ras/Raf/MEK/ERK, etc. This article aims at compiling the major signaling pathways involved in oral carcinogenesis. These pathways govern different hallmarks of oral cancer, like sustaining cell proliferation, resisting apoptosis, deregulating cellular energetics, genomic instability and mutations, inducing angiogenesis and metastasis/ invasion, avoiding immune destruction, stem cell population maintenance, tumor-promoting inflammation, etc. Comprehensive and robust research of these pathways in discovering new therapeutic or treatment strategies for oral cancer needs to be done. These pathways also act as prognostic markers of cancer and provide insight into various possibilities of new or improved inhibitors of oral cancer target proteins and genes. These pathways offer insight into a better understanding of oral cancer and are essential in new oral cancer management and treatment strategies.

Acknowledgements

The author would like to acknowledge the Defence Research and Development Organization (DRDO) for financial support (ERIP/202110007/M/01/1789).

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