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Oral Squamous Cell Carcinoma: The Molecular Characteristics

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Abstract. Oral squamous cell carcinoma (OSCC) is a malignancy that occurs on keratinocyte cells that have the potential to spread through lymphatic tissue or blood vessels. These carcinomas develop as a result of a series of molecular factors, which are influenced by individual genetic factors as well as environmental exposure to carcinogens. The evaluation of the prognosis and treatment plan for OSCC are mainly based on the clinical picture of the tumor or the TNM classification (T, tumor; N, lymph nodes or glands; M, metastases). The disadvantage of this diagnostic technique is that it does not consider the biological properties of the tumor, molecular characteristics, or tumor location, so it is deemed incapable of predicting patient safety. The purpose of this paper is to provide an overview of the etiology, pathogenesis, clinical picture, radiographic findings, and histopathological features of OSCC to help those concerned make the correct diagnosis. We use references from the last ten years article to obtain an up-to-date data. Based on our literacy study, we discovered that the presence of pseudoepitheliomatous hyperplasia (PEH) and keratoacanthoma distinguishes OSCC from many other oral cavity carcinomas (CA). The amount of P53 expression in tissues experiencing PEH and CA, as well as the integration of several other pro-cancer protein expressions, can be used to detect severity early. We can conclude that early detection of OSCC should be done by assessing the expression of proteins that cause the severity of cancerous lesions so that suppression of these cancer-inducing proteins can be treated while the levels are still low.

Keyword: OSCC, Molecular pathway, Biomarkers, prognosis, targeted theraphy

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1 Introduction

Cancer is the deadliest disease in humans and a major cause of morbidity. Cancer can be correctly identified as a malignancy once it has reached its final stage. When a patient receives therapy at a late stage, the efficacy of the therapy is reduced because the cancer-causing genes have spread and a nutritional supply has formed as a result of the cancer angiogenesis process

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[1]. Doctors and health analysts must understand the molecular changes from normal cells to hyperplasia, which do not show any changes in tissue morphology, and how changes in hyperplastic tissue lead to dysplasia, or what we call benign tumors, in order to detect cancer cases in patients early.

Years of genetic changes have resulted in the molecular pathogenesis of oral squamous cell carcinoma (OSCC) [2]. Proto-oncogenes code for growth factors, growth factor receptors, protein kinases, signal transducers, nuclear phosphoproteins, and transcription factors. Tumor suppressor genes do not control cell growth or differentiation, but their absence can lead to cancer [2]. Carcinogenesis is a multi-step process that begins with the loss of the 3p and 9p arms of the chromosome. The transformation of normal tissue into dysplasia characterizes this condition. Following that, several other chromosomal arms, including 8p, 13q, and 17p, are lost [3].

Loss of tumor suppressor genes is thought to occur at the 9p and 17p loci, where p16 and p53 are lost. LOH at 9p is found in 72% of lesions and is found at the coding site of the p16 cell cycle protein, which inhibits Cdk, allowing for excessive cell proliferation [4]. The process of chromosome-arm disruption can continue indefinitely, progressing from hyperplasia to dysplasia and finally to carcinoma in situ. In this literature review, we will evaluate some of the molecular changes in cancer cells in the squamous oral cavity from previous studies. To update information about the scope of the review, we only cite references from published articles published within the last ten years. Following the observation of cases of suspected OSCC in patients, it is hoped that readers will use this article as a guide for medical research or even treatment applications.

2 Histopathological of OSCC

The accumulation and interaction of genetic, environmental, hereditary, and behavioral factors can influence OSCC development and treatment response. Leukoplakia are histological representations of epithelial cell changes that lead to OSCC [5]. It begins with a thickening of the keratin layer on the surface epithelium (hyperkeratosis), with or without thickening of the lining's spinosum (acanthosis). Parakeratin (hyperparakeratosis), orthokeratin (hyperorthokeratosis), or both make up the keratin layer [2]. At the cellular and tissue levels, the progression from dysplasia to neoplasm is a sequential process (Figure 1). Differentiating dysplasia from in situ neoplasia is a common diagnostic challenge.

Neoplasia has progressed if there is cell anaplasia and loss of normal tissue structure. These changes are most commonly seen in the uterine cervical squamous epithelium, the oral squamous epithelium, the epidermis in sun-exposed skin layers, and the chronically inflamed colonic and gastric mucosa. Local mutagens and inflammatory mediators, as well as high cell turnover rates, all contribute to the accumulation of genetic disorders that lead to neoplasia [3].

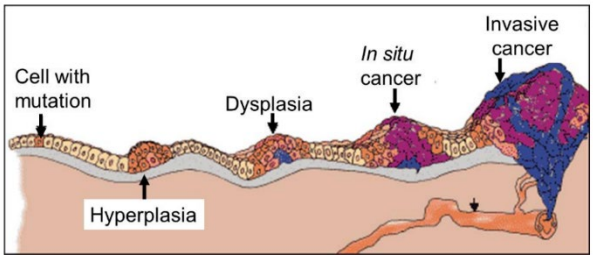

Figure 1. Dysplasia progresses to neoplasia [3]

Carcinoma in situ (CIS) is a type of pre-invasive malignant epithelial tumor that develops from squamous cells or glands. Because this cancer is still in its early stages, it does not invade the surrounding stroma or break or penetrate the local basement membrane. Carcinoma in situ can occur anywhere, including the cervix, skin, oral cavity, breast, and large intestine [6]. Grading is the histological evaluation of tumors that look like the normal tissue lining (squamous epithelium lining) and produce the same product (keratin). Rivera and Venegas [6] describe the characteristics of OSCC that set it apart from normal cells. Lesions are classified into three (I-III) or four (IV) grades (from I to IV). The lower the level of cell differentiation, the higher the grade value. In short, an OSCC that has matured enough to resemble the original tissue will grow slowly and eventually metastasize. This is referred to as "low-grade," "grade I," or "well differentiated" OSCC (Figures 2A and 2B). OSCC with cellular and nuclear pleomorphism and little or no keratin, on the other hand, have immature cells that make identification difficult at first. This type of OSCC grows quickly and spreads quickly; it is known as high-grade, or "poor differentiation," anaplastic carcinoma (Figure 2C). A "moderately differentiated" carcinoma is a tumor that falls between the two extremes (Figure 2D).

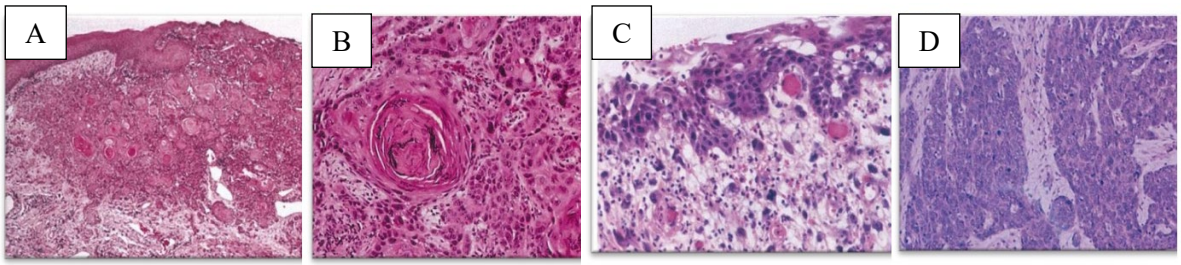

Figure 2. [A] The histopathological of OSCC “differentiation good”. Malignant squamous cells spread into the lamina propria due to the presence of epithelial islands. [B] Description histopathology of OSCC “differentiation good”. The presence of epithelial cell dysplasia with keratin pearl formation. [C] The histopathological of OSCC “badly differentiated”. A number of pleomorphic cells on the lamina propria shows anaplastic carcinoma. [D] “Moderately differentiated” OSCC. Although no keratinization is seen, the malignant cells can be easily distinguished from the normal squamous epithelial cells of their origin [6 and 7].

The evaluation of histopathological grading is a subjective process that is dependent on the available tumor samples and the pathologist's personal evaluation criteria. As a result, clinical staging outperforms microscopic grading in predicting prognosis. The majority of OSCC diagnoses are made through routine microscopic examination. Monoclonal antibodies against cytokeratins are needed for research to differentiate high-grade or poorly differentiated OSCC from other malignancies.

3 The Molecular Characteristic of OSCC

According to Nayak et al. [7], a typical image Microscopic OSCC contains pseudoepitheliomatous hyperplasia (PEH), which distinguishes it from other conditions such as inflammatory lesions, particularly on the gums. PEH is characterized by rete ridge elongation and pseudostromal invasion from squamous epithelium. Epithelial islands have irregular margins and heavily keratinized pearls. Keratoacanthoma (KA) is another symptom. Keratin pearls, lining, and epithelial islands are mitotic features of a squamous odontogenic tumor that pass into the stroma [8]. Squamous-odontogenic tumors are composed of mixed squamous epithelial islands with smooth margins. This island is causing central cystic and dystrophic calcifications to change. Carcinoma cuniculatum (CC) is a well-differentiated OSCC variant. CC is distinguished by the presence of branching, which results in papillary keratin projections. Stromal islands infiltrated widely and formed microabscesses [9]. How difficult is it? Knowing this characteristic picture alerts clinicians to lesions that exhibit unusual behavior, such as delayed healing and continuous enlargement accompanied by the presence or absence of bone destruction, necessitating a suitable biopsy technique.

3.1 OSCC Can Generate Enough Growth Signals

Exogenous growth signals are required for normal cells to proliferate. Cell surface receptors deliver this growth signal, which gradually activates intracellular signaling pathways, resulting in proliferation. There is an increase in growth factor receptors binding to their ligands during carcinogenesis, resulting in autocrine stimulation without exogenous factors. Increased expression of EGFR, TGF, and signaling proteins Intracellular signaling plays an important role in OSCC development [10]. The MAPK pathway is activated by the EGFR molecule, which includes PI3K, AKT, mTOR, Janus kinase (Jak), Signal Transducer and Activator of Transcription (STAT), and Protein Kinase C (PKC), all of which promote OSCC proliferation and survival, invasion, metastasis, and angiogenesis [11]. Hepatocyte Growth Factor (c-Met) is a receptor kinase that is overexpressed in OSCC and increases OSCC motility or spread via paracrine pathways that increase MMP-1 and MMP-9 expression [12]. Another growth signal is CycD1, a proto-oncogene that encodes a regulator of the positive G1 phase of the cell cycle that initiates DNA synthesis. A proto-oncogene that regulates cell growth and transmits mitogen signals from the cell surface to the nucleus is known as a Ras-oncogene.

3.2 OSCC Able to Avoid Apoptosis

Cell death, particularly apoptosis, is an important process because it not only describes disease pathogenesis but also provides clues on how to treat the OSCC. Anti-cancer drugs, graft versus host disease (GvHD), CD-4 cell death in Acquired Immunodeficiency Syndrome (AIDS), viruses that cause cell death such as Hepatitis B or C, radiation, hypoxia, cell degeneration such as Alzheimer's and Parkinson's, and death cells due to myocardial infarction are all pathological causes of apoptosis. Caspase activation, DNA and protein breakdown, and membrane changes that allow phagocytic cells to recognize them are the three main features of biochemical changes in apoptosis. Based on several integrated studies, the first stage of apoptosis is marked by the expression of phosphotidylserine (PS), which is thrown out from the inner layer to the outer layer of the cell membrane [13, 14]. The apoptotic bodies formed at the end of apoptosis allow macrophages to recognize dead cells without the release of cellular pro-inflammatory components [13–15].

Endonucleases break down internucleosomal DNA into oligonucleosomes of 80 to 200 base pairs during the final stage of apoptosis. Caspase activation is another feature of apoptosis. Caspase activation results in the release of essential cellular proteins as well as the breakdown of the nuclear scaffolding and cell wall framework. Another member of the Bcl-2 family is another apoptotic regulator. Based on a summary of several journals that have examined the apoptotic pathway of Bcl biomarkers in the last 10 years, there are 25 members of the Bcl-2 family that have been identified and classified into two groups based on their structural similarities [16]. Bcl-2 and Bcl-xL, which function as anti-apoptotic proteins, are members of the first group. Members of the second group include the pro-apoptotic proteins Bax and the Bcl-2 associated killer (Bak), as well as the novel BH3 domain-only death agonist (Bid) and the Bcl-2 associated death molecule (Bad) [16]. Apoptosis is classified into four stages based on the presence of death signals (apoptotic inducers) that can be physiological (hormones and cytokines), biological (viruses, bacteria, and parasites), chemical (drugs), or physical (radiation and toxins). The second stage is the integration or regulatory stage (signal transduction, induction of related apoptotic genes), followed by the apoptosis implementation stage, which involves morphological and chemical changes (DNA degradation, cell disassembly, formation of apoptotic bodies). Phagocytosis, or cell elimination by macrophages, dendritic cells, or cells adjacent to apoptotic cells, is the final stage. Apoptotic events include the condensation of the cell nucleus, the compaction and division of the cytoplasm into the apoptotic bodies' connective membranes, and the fragmentation of chromosomes into fragments containing various nucleosomes [17].

Another protein, a DNA endonuclease, is usually involved in the protein target. When the target protein is cleaved, the DNAase can move to the nucleus and start executing. when caspase-3 cleaves gelsolin, a protein that maintains cell morphology [16]. Gelsolin will cause the actin filaments in the cell to split. p21-activated kinase 2 is another protein required for the formation

of apoptotic bodies (PAK-2). Caspase-3 activates this kinase with limited proteolysis [17]. Caspase-3 also cleaves cytokeratin, particularly cytokeratin 18 (CK18), where a new epitope appears to be dominant during early apoptosis [18]. Apoptosis is induced by a variety of signaling pathways and regulated by a plethora of complex extrinsic and intrinsic ligands. There are two types of apoptotic pathways: those that involve caspase function and those that do not. Mitochondria function as "crosstalk" organelles, or organelles that participate in both apoptotic pathways.

Apoptotic signals mediated by caspases can occur both intracellularly and extracellularly. The extrinsic (extracellular) pathway is activated by stimulation of death receptors, whereas the intrinsic (intracellular) pathway is activated by the release of signaling factors from the cell's mitochondria. The extrinsic pathway of apoptosis begins with the release of signal molecules known as ligands by cells other than those that will undergo apoptosis. The ligand binds to death receptors on the target cell's transmembrane membrane, causing apoptosis. Based on several studies in the literature, the Tumor Necrosis Factor (TNF) receptor family, which includes TNF-R1, CD95 (Fas), and TNF-related apoptosis-inducing ligand (TRAIL)-R1 and R2, are cell surface death receptors [19, 20, 21]. When a ligand binds to the receptor, the initiator caspase 8 forms a trimer with the FADD adapter protein. TRAIL-R1 and TRAIL-R2 CD95 receptors bind to FADD, while TNF-R1 binds indirectly to another adapter molecule, TNF-Receptor Associated Death Domain (TRADD) [19, 21]. DISC refers to the complex formed between the receptor ligand and the FADD-Death-Receptor [22]. Intricately, this will activate pro-caspase-8, which will then activate the executioner caspase. Caspase-8 functions by cleaving a member of the Bcl-2 family, Bid. Bid will induce the insertion of Bax into the mitochondrial membrane and the release of proapoptotic molecules such as cytochrome c, Samc/Diablo, Apoptotic Inducing Factor (AIF), and OMI/Htr2. When dATP is present, a complex called the apoptosome is formed between cytochrome c, Apaf-1, and caspase-9. Caspase-3 flow will be activated by Caspase-9 [21].

Caspase-3 proteins actively degrade a variety of substrates, including DNA repair enzymes like poly ADP-ribose polymerase (PARP) and DNA protein kinases, which include cellular and nuclear structural proteins like the nuclear mitotic apparatus, nuclear lamina, actin, and endonucleases like inhibitors of caspase-activated deoxyribonuclease (ICAD) and other cellular constituents [23]. Caspase-3 can also activate other caspases, such as pro-caspase-6 and pro-caspase-7, which provide cellular damage amplification. Cellular stress increases the expression of the p53 protein, resulting in G1 arrest or apoptosis. Members of the Apoptosis Stimulating Protein p53 (ASPP) family, specifically ASPP 1 and ASPP 2, stimulate p53's transactivating function in the promoters of pro-apoptotic genes like Bax and p53 inducible gene 3 (PIG 3), but not in the promoters of cell cycle inhibitor genes like p21 and mdm2 [21].

Mitochondrial stress, which causes apoptosis in the intrinsic pathway, is caused by chemical compounds or a loss of growth factors, resulting in mitochondrial disruption and the release of cytochrome c from the mitochondrial intermembrane. Cytochrome c is a heme protein that functions as an electron carrier in mitochondrial oxidative phosphorylase, electron blocking in cytochrome c oxidase, and intermembrane exit binding to Apaf-1, a cytoplasmic protein [24]. In the cytoplasm, this protein will activate the caspase-9 initiator. Following electrochemical phosphorylation changes in the membrane, this protein exits the mitochondria.

The potential change creates a nonspecific channel in the permeable membrane that is made up of two inner membrane proteins called Adenine Nucleotide Translocator (ANT) and two outer membrane proteins called porins and Voltage Dependent Anion Channel (VDAC). These proteins may interact on the outside, and there is contact on the inside. Substances with a molecular weight of less than 1500 can pass through this channel. Changes in the proton gradient cause mitochondrial oxidation and phosphorylation, while changes in ionic strength cause matrix swelling [25]. The inner side is highly wrinkled and has a significantly larger surface area than the outer membrane. As the matrix swells, the outer side is damaged, allowing cytochrome c and Apaf-1 to move in and out of the cytoplasm.

In addition, other lethal stimuli, such as DNA damage, oxidative stress, and hypoxia, commonly activate this pathway. Proapoptotic factors such as cytochrome c and AIF are found in the mitochondria. When these are released into the cytoplasm, they activate the caspase activation pathway. Its release is controlled by mitochondria-bound Bcl-2 families, specifically Bax and Bad. The inner mitochondrial membrane allows cytochrome c to enter the cytoplasm by passing through pores in the outer membrane, a study has shown. The Caspase Recruitment Domain is formed when cytochrome c is released into the cytoplasm and binds to Apaf-1 (CARD) [26]. The apoptosome complex is formed by the combination of several CARDs, which then bind to pro-caspase-9 and activate it into caspase-9 (the initiator caspase). Caspase-9 will activate procaspase-3, transforming it into caspase-3, an effector caspase that causes apoptosis [27]. Caspase is a key protein in the cell signaling pathway that leads to cell death when activated by another caspase. Caspases are widely expressed as proenzymes (zymogens) and are activated by other proteases via autoproteolytic mechanisms, resulting in their release. Table 1 lists the different types of caspases.

Caspase inhibition derived from a family of apoptosis inhibitors (IAPs) such as survivin, cIAP-1, cIAP-2, ILP-2, XIAP, livin, BIRC, and NAIP maintains the balance of caspase action. The IAPs family can inhibit initiator and executor caspase through a variety of mechanisms. Caspase-8 activity is regulated by FADD-like ICE (FLICE)-inhibitory proteins (FLIPs) [28]. Through similar interactions, this protein can bind FADD and caspase-8, inhibiting caspase-8 from forming DISC. Figure 3 summarizes the extrinsic and intrinsic pathway mechanisms.

Table 1. Caspases that are involved in apoptosis

Cysteinyl aspartic acid-protease (Caspase)		
Type	Name	Synonim
Initiator (apical)	Caspase-2	ICH1, Nedd2
	Caspase-8	FLICE, MACH1, MCH5, FADD□like ice
	Caspase-9	MCH6, ICELAP6
	Caspase-10	FLICE2, MCH4
Effector (Executor)	Caspase-3	Caspase-3
	Caspase-6	MCH2
	Caspase-7	MCH3, CMH, ICELAP3
Inflammation	Caspase-1	ICE
	Caspase-4	ICH2, TX, ICErll
	Caspase-5	ICErlll, TY
	Caspase-11	-
	Caspase-12	-
	Caspase-13	Caspase-13
	Caspase-14	MICE

Granzyme A (GzmA) can directly increase reactive oxygen compounds and mitochondrial damage via the independent caspase pathway. The 270–420 kDa endoplasmic reticulum (ER)-associated complex containing GzmA-activated DNase NM23-H1 or the SET complex are Gzma-specific targets. The SET complex will enter the nucleus and damage the DNA. AIF, which is released by mitochondria and causes DNA damage in the nucleus, is the most important pro-apoptotic factor in this pathway. Because of the presence of reactive oxygen compounds, PARP-1 activation influences AIF release by mitochondria. This demonstrates that reactive oxygen compounds are involved in both caspase-dependent and caspase-independent pathways. Aside from AIF, glutathionylation and nitrosylation inhibit several thiol groups and affect protein function, resulting in apoptosis. Figure 4 depicts the independent caspase pathway mechanism.

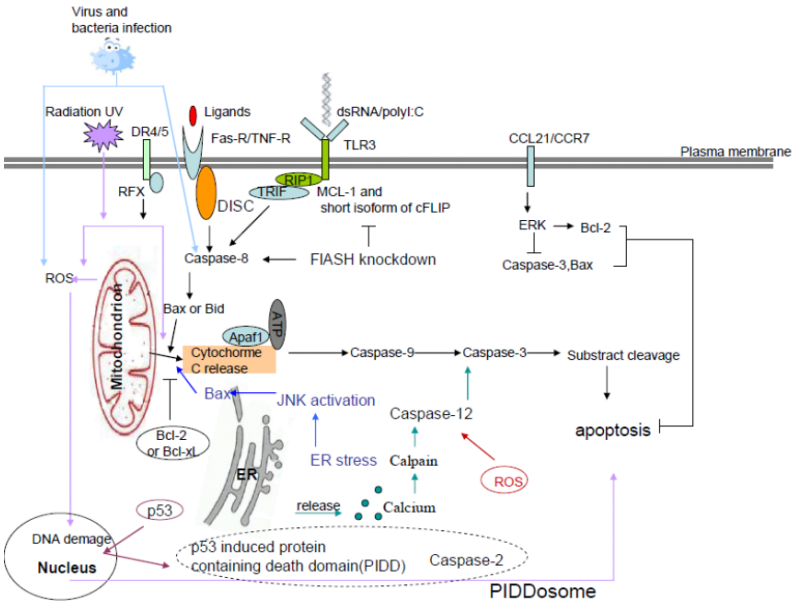

Figure 3. Caspase-dependent (extrinsic and intrinsic) pathways. Mitochondria and nuclear organelles are crucial in this type of apoptosis. These organelles can connect various caspase activation signals, causing changes in reactive oxygen compounds, cytochrome c, and mitochondrial membrane potential. External ligands, in addition to the mitochondrial pathway, can activate ERK, which is followed by a cascade of caspase activities.

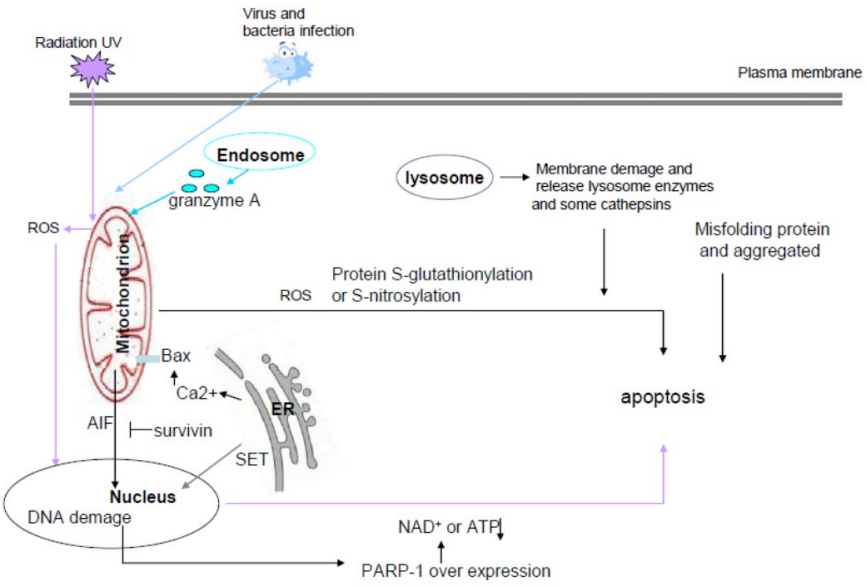

Figure 4. Caspase-independent pathway. This type of apoptosis does not involve caspases and thus cannot be inhibited by caspase inhibitors. AIF, reactive oxygen species, Ca2+, ATP, protein modification and misfolding, and DNA damage are all examples of cell components that can cause caspase-independent apoptosis.

3.3 OSCC Avoidance of Apoptosis

Apoptosis avoidance during carcinogenesis is important in cancer development. When this homeostatic balance is disrupted, the population of clonal mutation cells increases, leading to the development of tumors. Active mutations in oncogenes or inactive mutations in tumor suppressor genes initiate cell division (initiation). The mechanism of apoptosis avoidance in carcinogenesis is divided into three major events: disruption of the balance of pro and anti-apoptotic proteins, decreased caspase function, and disruption of death receptor signaling [15]. Aside from these three events, p53 mutations and increased IAP expression influence apoptotic activity in OSCC [29]. Figure 5 depicts this mechanism.

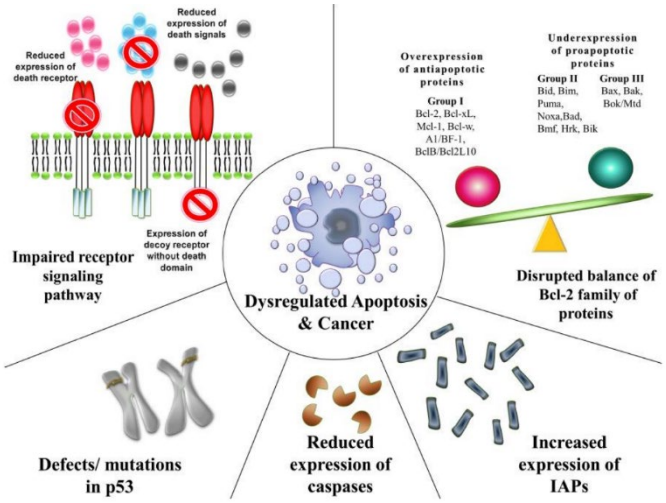

Figure 5. Mechanisms affecting apoptosis and cancer

3.4 Loss of Balance between Pro-apoptotic Proteins and Anti-apoptotic

The issue in OSCC related to the activity of pro- and anti-apoptotic proteins is not one of quantity, but rather of changes in the ratio between these two proteins, which play an important role in cell death. This condition is also influenced by gene expression. One of the proteins that influences it is from the Bcl-2 family. This protein is involved in many aspects of the mitochondrial intrinsic pathway. The Bcl-2 protein is the second member of the B-cell lymphoma protein family. It is found in the mitochondrial membrane's outermost layer. If the balance of anti- and pro-apoptotic proteins is disrupted due to over- or under-expression, apoptosis will be dysregulated. Juneja et al. [30] reported that impaired expression of Bcl-2 at the terminal differentiation stage of keratinocytes inhibited apoptosis in OSCC. This protein's increased expression protects tumor cells from apoptosis. Fulda et al. also discovered an increase in Bcl-2 expression to prevent TRAIL-induced apoptosis in neuroblastoma, glioblastoma, and breast cancer.

The p53 gene is the "Guardian of the Genome" and plays an important role in cell cycle regulation, development, differentiation, gene amplification, DNA recombination, DNA segregation, and cellular senescence. Defects in the p53 tumor suppressor gene have been found in over half of all cancer cases. Rawda et al [31] discovered p53 expression in 86% of cases of premalignant lesions above the basal cell layer that could develop into OSCC. On immunohistochemical examination, Mardanpour et al [32] demonstrated that the presence of epidermoid carcinoma metastases to lymph nodes, larger tumor size, and poor prognosis had a significant correlation with increased expression of p53 and Ki-67. Zaid et al [33] found a 74%-78% increase in immunohistochemical expression of the p53 gene in OSCC and dysplastic lesions in cigarette addicts.

In addition to the Bcl-2 and p53 families, IAPs are proteins that regulate the balance of apoptosis, cytokinesis, and signal transduction. The presence of the Baculovirus IAP Repeat

(BIR) protein domain triggered the appearance of this protein [34]. There are currently eight IAPs known: NAIP (BIRC1), c-IAP1 (BIRC2), c-IAP2 (BIRC3), X-linked IAP (XIAP, BIRC4), Survivin (BIRC5), Apollon (BRUCE, BIRC6), Livin/MLIAP (BIRC7), and IAP-like protein 2 (BIRC8) [35]. These proteins are endogenous caspase inhibitors that work by binding to the BIR domain of the caspase's active site, resulting in caspase degradation or removal from its substrate. IAPs regulation disruption has been widely reported in several cancer cases. Mohammad et al [36] discovered an abnormal expression of the IAP family in pancreatic cancer, as well as a proclivity for chemotherapy resistance. Survivin, another type of IAP, is closely related to OSCC. Survivin expression in high-risk patients can be used as a marker of aggressive OSCC.

3.5 Decreased Caspase Activity

Caspases are broadly classified into two types: those related to caspase-1 and those involved in cytokine release during the inflammatory process (caspase-1, 4, 5, 13, and 14). The second group is the one that plays the most important role in apoptosis (caspase-2, 3, 6, 7, 8, and 9). This second group is divided into two parts: initiator caspases (caspase-2, 8, 9, and 10) that participate in the early apoptotic pathway and effector caspases (caspase-3, 6, and 7) that participate in cellular component leakage during apoptosis [27]. In OSCC tumor tissue, Poomsawat et al. [37] found a decrease in zymogen caspase-3 activity and an increase in survivin expression. Those studies describe survivin, which inhibits caspase-3 synthesis and activation, preventing OSCC cell apoptosis. Li et al. [38] used tissue microarray technology to look at immunocaspase expression. Caspases 8 and 9 are found in abundance in the OSCC, but caspases 3, 6, 7, and 10 appear only infrequently. Researchers discovered caspase-7 to be an independent prognostic factor in OSCC patients. Reduced caspase function can reduce apoptosis and promote carcinogenesis. Other research has found a caspase-3 mRNA deficiency in RNA samples from breast, uterine, and cervical cancers [39].

3.6 Disruption of Death Receptor Signaling

Death receptors and death receptor ligands are important in the extrinsic pathway of apoptosis. This receptor has a death domain that, when activated by a death signal, attracts a number of molecules, activating a series of signals for caspase release. Several abnormalities in death receptors have been discovered, including decreased expression of surface receptors [40], which can lead to drug resistance. Drug resistance in leukemia and neuroblastoma cells can be caused by decreased CD95 expression [41]. According to Contreras et al. [42], loss of Fas and impaired regulation of FasL, DR4, DR5, and TRAIL in cervical intraepithelial neoplasia can accelerate carcinogenesis [42].

3.7 OSCC are Not Sensitive to Inhibitory Signals Growth

A. Cell Cycle Regulation

The cell cycle starts when cells move from the G0 (quiescent) phase to the G1 phase as a result of growth factor stimulation. The Cdk4/6 complex with CycD initiates phosphorylation of the retinoblastoma family of proteins during early G1 (pRb). Rb is required for G1 phase regulation and influences Cdk phosphorylation. Early in the G1 period, pRb is found in hypophosphorylated concentrations and binds tightly to the transcription factor E2F, suppressing its action. At the CDK attachment site, it becomes hyperphosphorus-lylated, allowing transcription to occur [43]. Cells with this condition can pass through the restriction points at the end of the G1 phase and enter the S phase. This phosphorylation activity is a continuous series of processes initiated by CycD-related Cdk4 and Cdk6 [44]. The subsequent effect of phosphorylation on CycE disrupts the relationship between pRb and deacetylated histones, which should keep the chromatin structure compact or cause chromatin reformation. The DNA structure loosens, the transcription factor that was originally bound to pRb is released, and transcription of the E2F-responsive gene required for cell cycle progression to S-phase becomes active. This cycle is known as the RB Pathway.

The cell cycle begins with the entry of cells from the G0 (quiescent) phase into the G1 phase as a result of growth factor stimulation. During early G1, the Cdk4/6 complex with CycD initiates phosphorylation of the retinoblastoma family of proteins (pRb). The Rb protein is essential for G1 phase regulation and influences Cdk phosphorylation. pRb is found in hypophosphorylated concentrations early in the G1 period and binds tightly to the transcription factor E2F, suppressing its action. At the CDK attachment site, it becomes hyperphosphorus-lylated, allowing transcription to occur [43]. Cells with this condition can pass through the restriction points at the end of the G1 phase and enter the S phase. This phosphorylation activity is a continuous series of processes initiated by CycD-related Cdk4 and Cdk6 [44]. The subsequent effect of phosphorylation on CycE disrupts the relationship between pRb and deacetylated histones, which should keep the chromatin structure compact or cause chromatin reformation. This causes the DNA structure to loosen, the transcription factor that was originally bound to pRb to be released, and the transcription of the E2F-responsive gene required for cell cycle progression to S-phase to become active. The RB Pathway is the name of this cycle.

CycE will be degraded as the cell cycle approaches S-phase, and the liberated Cdk2 will bind CycA. During the S-phase, cells require the Cdk2-CycA complex to replicate DNA. The Cdk2-CycA complex will phosphorylate active DNA replication proteins such as the Cell Division Cycle 6 protein (Cdc6) [48]. Additionally, the complex inhibits DNA replication and multiplication. At the end of the S phase, CycA releases Cdk2 and binds to Cdk1 (Cdc2), controlling the cell's transition from S to G2. The CycA-Cdk1 complex will aid in the condensation of chromatin, which is required for cell proliferation. If there is an error in DNA synthesis, the cell has the opportunity to repair itself when it enters the G2 phase [46, 47]. If the

cell ensures that the DNA is duplicated correctly and the chromosomes are separated correctly, the cell cycle performs a checkpoint mechanism. Checkpoints are responsible for detecting DNA damage. If the checkpoint detects DNA damage, it will either activate a temporary cell cycle barrier for DNA repair or a permanent barrier, causing the cell to enter the senescent phase. If the cell cycle inhibition mechanism fails to ensure that the damaged DNA is duplicated, the cell will die via apoptosis. Figure 6 depicts the cell cycle phases.

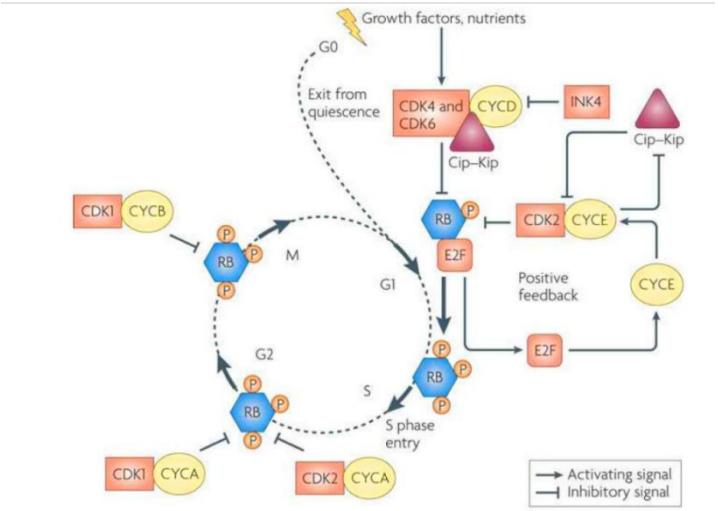

Figure 6. The cell cycle. This cycle consists of phases G1 (presynthesis), S (synthesis of DNA), G2 (premitosis), and M (mitosis). Quiescent (resting state) is in the G0 phase.

The first checkpoint factor, R, appears near the end of G1 and determines whether the cell is ready to begin DNA synthesis. When DNA is damaged, the cell cycle is stopped until the damaged DNA repair mechanism is finished. Cancer will result if the double-chain damage is successful in destroying the gene's integrity. The p53 gene product is critical for maintaining the G1/S phase checkpoint. This gene protein acts as a receptor for stress signals, such as DNA damage. The p21 gene, which is induced by p53, binds to Cdk2/CycE, causing cell cycle arrest [49] (Figure 7).

DNA damage was able to induce faster p53 activity, which is regulated by mdm2, a degrader of p53, than previously thought, according to a study published in the journal Cell Stem Cell Reports [50]. However, DNA damage could also increase p53 activity. The genes Mdm2 and p19ARF regulate the p53 and cyclin genes, respectively. Once activated, protein levels rapidly rise over their half-lives, while mRNA levels remain relatively constant. Ubiquitin binding to the E3 ligase on mdm2 can cause proteolytic degradation of the p53 gene [51]. The p53 gene is activated, causing mdm2 levels to rise. Ataxia Telangiectasia Mutated (ATM) and DNA-dependent protein kinase (DNA-PK) are activated by DNA damage and can phosphorylate critical serine residues in p53's mDM2 binding domain.

B.

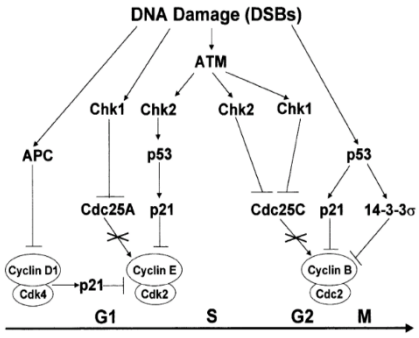

Figure 7. Checkpoints due to DNA damage in the G1/S and G2/M phases.

Normally, the p53 protein is present in low concentrations in cells. Protein levels rapidly rise after activation, whereas mRNA levels remain relatively constant. DNA damage can also result in increased p53 activity, which is controlled by mdm2 and p19ARF [31-33]. mdm2, a p53-responsive gene, is a p53 negative regulator (a gene expressed via the transcription factor p53). Mdm2 will inactivate p53 by binding to it or degrading it. One of the factors that can inhibit mmndre is the stimulation of DNA-damaging agents. Mutations in the ATM-RAD3 gene increase sensitivity to x-rays and tumor incidence [52]. The p53 gene detects DNA damage in a cell and either stops the cell cycle so that the cell can repair the damage or, in many cases, simply tells the cell to commit suicide. Oncogenes and DNA-damaging agents both activate p53 in different ways [53]. The p53 gene detects DNA mutations and shuts down the cell cycle. The oncogene does not activate ATM or DNA-PK, but instead inhibits mdm2 by increasing the expression of p19ARF, a tumor suppressor protein. This is yet another method of inhibiting mDM2 [54].

ATM, Nibrin, Mre11, and Rad50 are proteins that play numerous roles in this phase in mammalian cells. These four proteins are also highly dependent on pRb dephosphorylation to stop the cellular cycle when DNA damage occurs [55]. The G2 checkpoint ensures that the cell is ready to enter the M phase and prevents mitosis from starting before DNA replication is complete. This checkpoint function is also responsible for keeping Cdc2/cyclin B1 inactive. Chk1 is the primary checkpoint protein in the S/G2/M phase and phosphorylates Cdc25A, inactivating the Cyc-Cdk complex and causing S arrest [56]. The final checkpoint, known as the spindle checkpoint, is in charge of maintaining the genome's integrity toward the end of mitosis. Mitotic termination occurs if the chromosome pair is not properly positioned on the spindle. These checkpoints do not function properly in cancer cells, allowing the cell cycle to continue unabated.

C. Regulation of the OSCC Cycle

Changes in the genetic control of cell division, which lead to uncontrolled cell proliferation, are fundamental changes in cancer. Mutations in the Cdk1 and Cdk2 genes occur in colonic

adenomas, and overexpression of these two genes can result in adenomatous tissue focal carcinoma. CycD functions as a growth sensor, connecting mitogenic stimuli to the cell cycle [57]. Translocation of the CycD1 gene has been linked to parathyroid adenoma, a B lymphocyte malignancy that includes mantle cell lymphoma. CycD2, D3, and E overexpression can result in acute lymphoblastic and myeloid leukemia. Cdc25A regulates the G1/S transition and activates Cdk1-B during mitosis. CKI inhibitory activity can cause tumors in addition to interfering with CDK [44, 47]. The p16INK4 gene has been found to be missing in 50% of gliomas, mesothelioma, and 40–60% of nasopharyngeal, pancreatic, and bile duct tumors. The p16INK4 gene inhibits proliferation by binding to Cdk4 and Cdk6 and preventing cells from entering the S phase [46]. In each type of cancer, the p53 gene is the most frequently mutated. HPV E6 and adenovirus E1B-55K can both alter or inhibit p53 function. The p21WAF1 gene encodes a cell cycle inhibitor that is activated by both normal and mutant p53. Cell cycle inhibition is caused by the p21 gene's interaction with Cdk [58].

D. Ki-67 Protein as a Proliferation Marker

Ki-67 is a nucleolar protein that can be identified using monoclonal antibodies. This protein is required for cell cycle development in both human and mouse cells, but its molecular function is still unknown. Ki-67 protein is a marker of cell proliferation. Growth fraction markers, cell cycle-specific phase markers, and cell cycle time markers are the three types of proliferation markers [59]. Ki-67 is found in many nucleoli, particularly in the periphery, when a stage occurs during interphase in proliferating culture cells. It is scattered in small amounts in the nuclear foci during the early G1 phase and gathers around the formed nucleolus. Ki-67 appears in the M phase during hyperphosphorylation and differs in its DNA binding from the interphase phase [59]. Ki-67 is found in the nucleolus, a prominent structure in the nucleus, and becomes more concentrated as the cell progresses into S-phase. Ki-67 also moves from the nucleus to the condensed chromosomes and then to the chromosomal periphery during the prophase phase [60].

Hyperproliferation is an early indicator of growth problems. Proliferating Cell Nuclear Antigen (PCNA), Ki-67, CycD, and Centromere Protein F are common immunohistochemical markers (CENP-F). Proliferation increases in proportion to the severity of the lesion, and cell regulatory mechanisms are impaired or dysfunctional during the multi-step process of carcinogenesis [61]. Ki-67 is thought to be important in predicting the prognosis of oral cancer patients. Ki-67 appears during the active phase of the cell cycle's mitotic process (G1, S, G2, and M), and peak concentration occurs during the G2 and M phases. It is now commonly used in research because it is easier to color and produces good interpretation results [59].

E. OSCC cells have an unlimited and immortal replication potential.

Telomeres are the ends of long stretches of DNA. They are included in DNA strands but do not code for any proteins, so they are not classified as genes. Telomeres play an important role in

the stability of each cell's genome. Tumorigenic cells become immortal and able to divide as their telomeres lengthen [62].

When a cell divides, the entire DNA strand that makes up the genome is affected. The cell will go through several stages before it reaches the S phase. This is the stage where the genetic code doubles at the ends of each individual strand. If a cell lacks the telomerase enzyme, it cannot duplicate the very end of its DNA strand but can still divide. As a result, the DNA strands in the daughter cells are shorter than those in the mother cells, resulting in increased cell division rates [63]. If a cell's DNA strands become too short, the genome's stability is compromised. This may trigger the cell suicide program or cause the cell to stop dividing and enter senescence. Because cancer cells have a high number of telomerase enzymes, replication occurs continuously, even if replication is imperfect.

3.8 OSCC Capable of Inducing Angiogenesis

Another feature of OSCC development is angiogenesis. If the OSCC does not receive adequate blood supply from the body, it will grow slowly, reaching only 1-2 mm3 in size [1]. Solid tumors require a blood supply to grow and spread. The balance of pro- and anti-angiogenic factors determines the ability of these cancer cells. Vascular Endothelial Growth Factor (VEGF) [64], Platelet-derived Growth Factor (PDGF) [65], acidic and basic fibroblast growth factors (FGF 1 and 2) [66], and IL-8 are pro-angiogenic factors [67]. Interferons, proteolytic fragments such as angiostatin and endostatin, and thrombospondin-1 are all negative regulators of angiogenesis [1, 64, 65, 66, 68].

3.9 OSCC with the Ability to Invade Tissues and Metastasize

Local invasion and spread to the cervical lymph nodes distinguish OSCC. Cell adhesion, cytoskeletal rearrangement, cell migration and basement membrane dissolution, intravasation, cancer cell survival in the bloodstream, extravasation to distant sites, and distant growth of OSCC are all processes involved in OSCC invasion and metastasis. This ability emerges as a result of neo-angiogenesis stimulation. A process known as mesenchymal-epithelial transition occurs during invasion and metastasis. This transition is a process that causes increased migration, loss of epithelial integrity, local invasion, reorganization of the cell framework, redistribution of organelles, and changes in the gene expression profile of OSCC by changing the phenotype of polarized epithelial cells to phenotypic forms like mesenchymal fibroblast cells [69].

4 Conclusion

Much has changed in our understanding of the molecular pathogenesis of oral cancer over the last few decades. OSCC is a multistep process that necessitates the accumulation of genetic changes influenced by tobacco, alcohol, and possibly viruses in the context of potentially hereditary cancer mutagens. The two main targets of these genetic changes are the inactivation

of tumor suppressor genes and the activation of oncogenes. In the absence of extracellular growth stimuli, the combination of these two factors increases growth factor or cell surface receptor production, and activation of intracellular signaling messengers leads to tumor cell autonomy. Understanding the disease's molecular pathogenesis will allow researchers to develop appropriate therapeutic targets for cancer cures.

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