Hypermethylation of Apoptotic Genes in Oral Squamous Cell Carcinoma

ABSTRACT

Oral squamous cell carcinoma (OSCC) presents a substantial worldwide health challenge due to its aggressive behavior and resistance to standard treatments, resulting in high mortality rates. Epigenetic changes, notably DNA hypermethylation, are pivotal in OSCC development as they silence tumor-suppressor genes and drive tumor growth. This review highlightsthe hypermethylation status of apoptotic genes in the promoter region of CpG island which results in the formation of OSCC.This article utilizes different scientific databases such as Google Scholar, PubMed, NCBI, etc. to understand the interplay between external factors and epigenetic modifications which provides valuable insights for preventive strategies and personalized approaches to OSCC.The review discusses the epidemiology and causes of OSCC, emphasizing its resistance to therapy and poor prognosis. It also assesses current treatment strategies targeting DNA hypermethylation, such as inhibitors of demethylation and histone deacetylase, for their potential to improve patient outcomes. Future research directions center on investigating combination therapies targeting various epigenetic regulators and creating non-invasive methods for early cancer detection and prognosis evaluation. Overall, this review underscores the significance of DNA hypermethylation in OSCC progression and highlights the therapeutic promise of addressing epigenetic changes, especially those affecting apoptotic genes, to enhance patient survival.

Keywords: Hypermethylation, Apoptotic genes, Cancer, Epigenetics.

1. INTRODUCTION

Cancer, a diverse and intricate set of conditions, presents itself through the unregulated multiplication of cells, leading to the potential invasion of nearby tissues and the prospect of spreading to distant organs. The worldwide significance of cancer is considerable, marked by the diagnosis of millions of new cases annually. Among non-communicable diseases (NCDs), cancer ranks as the second principal contributor to mortality, following cardiovascular disease [1]. A tumor refers to an irregular cell proliferation that can be classified either as malignant or benign. Benign tumors like skin warts, are limited to a specific area and do not attack neighboring tissues or metastasize to distant organs. In contrast, malignant tumors have the potential to invade nearby tissue and spread through the bloodstream or lymphatic system. Only malignant tumors are considered cancers due to their invasive and metastatic nature, posing a significant threat to health. While surgical removal is often effective for benign tumors, the metastasis of malignant tumors renders them resistant to localized therapies. Tumors, whether benign or malignant, are categorized according to their cellular origin.

OSCC, a form of oral tumor, constitutes more than 90% of all malignant cases in the oral region and contributes significantly to the overall incidence of tumors in the head and neck region, accounting for 38%. Its onset is marked by a gradual accumulation of genetic alterations and tumor evasion of the host immune response [2]. The abnormal proliferation of malignant tissue is widespread across various regions of the oral cavity, including the labial mucosa, floor of the mouth, gingiva, palatal area, vestibule, buccal mucosa, alveolar ridge, and tongue. Notably, the distribution pattern of OSCC cases reveals a higher prevalence in certain regions, with approximately 32% occurring in the buccal mucosa, 11% in the lower lip, 22% in the tongue, 11% in the palate, 5% in the floor of the mouth, 3% in the gingiva, 8% in the vestibule, and 5% in the alveolus.

In 2020, a report by GLOBOCAN showed 377,713of new oral cancer caseson a global level, which resulted in approximately 177,757 ofdeaths in ayear. In India, it was observed that there were 135,929 of new cases, and 75,290 ofdeaths were reported each year based on the available data. Oral cancer is notably prevalent, particularly in Asian countries, constituting approximately fifty to seventy percent of all cancer-related fatalities in India [3]. The progression of oral cancer involves various stages influenced by both internal and external factors. There has been a notable rise in the incidence of oral cancer in certain regions, including India, Sri Lanka, Bangladesh, Taiwan, and Pakistan, collectively contributing to approximately 25% of newly reported cases. Notably, OSCC demonstrates a male predominance among affected populations [4]. Despite advancements in therapeutic modalities such as radiation, surgery, and chemotherapy, treatment-resistant OSCC remains a significant challenge. The 5-year survival rate for individuals with OSCC has persistently hovered around 50% for an extended period, indicating ongoing obstacles in achieving substantial improvements [5]. Projections suggest a tripling of cancer incidence by 2030, despite extensive research efforts currently underway. OSCC is characterized as a highly aggressive form of cancer, displaying considerable variability in etiological, clinical, and molecular aspects [6].

Significant contributors include consistent consumption of tobacco and alcohol, along with persistent infection of human papillomavirus (HPV) [7]. These risk factors may trigger various genetic and epigenetic pathway which enables the development and metastasis of tumors in addition to genomic instability. Oral premalignancy conditions and OSCC arise from genetic alterations, encompassing permanent modifications such as deletions, amplifications, and mutations in DNA sequence that initiate the activation of oncogenes or the suppression of tumor suppressor genes (TSGs). Epigenetics, as defined by Cavalli and Heard (2019), investigates the molecules and processes that maintain distinct patterns of gene activity while preserving the same DNA sequence [8]. Environmental and lifestyle factors interact with genetic information, significantly influencing genome activity. Epigenetic mechanisms produce various adaptable structural configurations that influence gene expression independently of genomic alterations. These mechanisms involve coordinated adjustments to DNA and chromatin which are classified into various types such as histone alterations, DNA methylation, and regulatory small non-coding RNAs. Epigenetic mechanisms could also contribute to the silencing of Tumor-SuppressorGenes [9]. Additionally, alterations caused by epigenetic changes contribute to this process which involves many gene expression variations that occur without any changes in DNA sequence. Changes in epigenetics can have a significant impact on metastasis, chemotherapy response, and tumor progression [10].
At present, three primary forms of epigenetic mechanisms are identified: histone modification, ribonucleic acid interference (RNAi), and DNA hypermethylation. Any disruption in these interdependent epigenetic mechanisms results in aberrant gene expression, which can cause cancer and other “epigenetic diseases” [11]. Methylation, a prevalent DNA modification in eukaryotic organisms, has garnered considerable attention owing to its capacity that influence gene expression. Alterations in the pattern of methylation may lead to either hypomethylation or hypermethylation. Hypomethylation of DNA is linked to gene reactivation and chromosomal instabilities [12-13]. On the contrary, gene repression is associated with hypermethylation in the promoter regions of the genes [14]. The hypermethylation of DNA, which results in the silencing of genes, affects a multitude of genes related to various cellular pathways. This includes functions like suppressing tumors, repairing DNA, responding to hormones, adhering to cells, and metabolizing drugs, among other roles [15].

2. EPIGENETIC REGULATION OF GENE EXPRESSION: FOCUS ON DNA METHYLATION

DNA methylation is an inheritable epigenetic modification characterized by the addition of a methyl group to cytosine bases within DNA molecules, facilitated by enzymes known as DNA methyltransferases (DNMTs), as illustrated in Figure 2. In mammals, DNA methylation primarily targets cytosine residues within various genomic contexts and is one of the extensively studied forms of chromatin modification. In somatic cells, the majority of DNA methylation occurs within CpG dinucleotide sequences, constituting over 98% of total methylation. However, in embryonic stem cells, a significant proportion of methylation occurs outside CpG contexts, accounting for up to a quarter of total methylation. DNA methylation is regulated by a group of enzymes called DNA methyltransferases (DNMTs) [16-17].

DNA methylation is essential for normal developmental processes and significantly influences critical mechanisms such as X-chromosome inactivation, genomic imprinting, and the regulation of repetitive element expression and movement. Dysregulation of DNA methylation is implicated in pathological conditions, including cancer [18]. Hypomethylation induces instability in chromosomes and the reactivation of silenced genes, including proto-oncogenes thus enhancing cancer progression. Hypomethylationis also responsible for contributing to oral carcinogenesis by resulting in loss of imprinting due to which alteration in the expression of genes occurs.Similarly, hypermethylation in the promoter region leads to the inactivation of TSGs, disrupts DNA repair mechanisms, and facilitates evasion of the immune system. Furthermore, numerous hypermethylated genes have been identified that could potentially impede OSCC progression in certain instances. These epigenetic alterations serve as fundamental molecular events in OSCC tumorigenesis, offering promising avenues for diagnostic as well as therapeutic interventions [19] (Fig. 1).

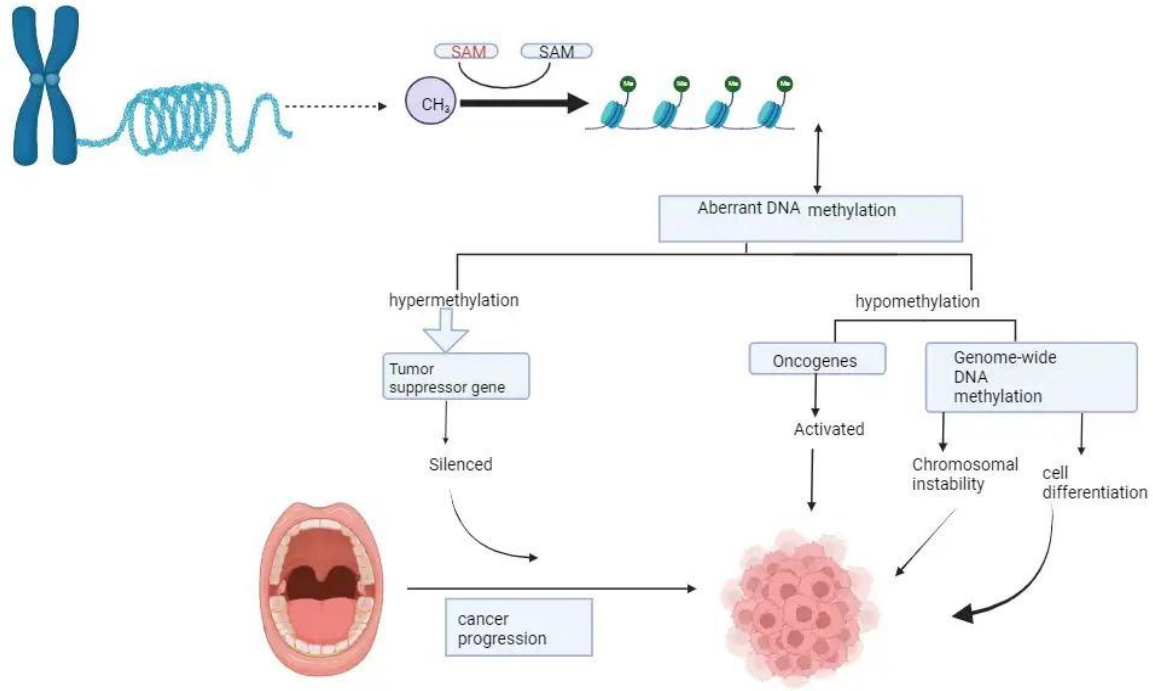

Fig. 1. Schematic diagram of aberrant DNA methylation pattern. Diagram showing the impact of both Hypermethylation and hypomethylation in OSCC progression. Hypermethylation of tumor-suppressor genesis responsible for their inactivation, restricting their ability to control cell growth and suppression of tumor development. Conversely, hypermethylation inhibits the transcription of cell division-inhibiting genes, such as oncogenes. Genome-wide DNA hypomethylation induces chromosomal instability and gene mutations while also contributing to normal cell function and differentiation. Understanding these patterns of DNA methylation is essential for comprehending the underlying mechanisms of cancer development.

CpG islands frequently display hypermethylation in tumor regions, leading to the transcriptional suppression of TSGs and promoting cancer progression. Conversely, hypomethylation or demethylation of CpG islands has been observed to reduce proto-oncogene transcription, resulting in chromosome instability, which is an early characteristic feature of tumorigenesis [20].

3. APOPTOSIS

Apoptosis is a controlled cellular process designed to preserve cellular balance and eliminate impaired cells. Cells undergoing apoptosis display distinct characteristics, such as condensed nuclei, fragmented DNA, membrane blebbing, and heightened cell membrane permeability [21-22]. It encompasses three primary pathways: intrinsic, extrinsic, and granzyme B pathways.

Regardless of the pathway involved, the outcome is the activation of caspase proteins, initiating a cascade of proteolytic events responsible for breaking down and eliminating the dying cell [23]. This process serves as one of the primary defenses against the development of cancer since cancer is characterized by resistance to cell death. It is also an essential part of normal cell turnover and tissue homeostasis [24].

3.1. Intrinsic pathway

The mitochondrial pathway, also known as the intrinsic pathway of apoptosis, is a key mechanism by which cells undergo programmed cell death in response to internal signals, such as cellular stress, DNA damage, or developmental cues. This pathway is initiated by perturbations within the cell itself rather than external stimuli. The process begins with the activation of pro-apoptotic proteins, particularly Bax and Bak, which are members of the Bcl-2 protein family. These proteins undergo conformational changes and oligomerization, leading to the formation of pores in the mitochondrial outer membrane. This results in the release of pro-apoptotic factors from the mitochondrial intermembrane space into the cytoplasm, including cytochrome c. Once released into the cytoplasm, cytochrome c interacts with the apoptotic protease activating factor 1 (Apaf-1), leading to the formation of a large multimeric protein complex known as the apoptosome. Within the apoptosome, procaspase-9 molecules are brought into proximity and undergo autocatalytic cleavage, resulting in the activation of caspase-9. Activated caspase-9 serves as an initiator caspase, triggering a cascade of caspase activation. Caspase-9 cleaves and activates downstream effector caspases, such as caspase-3 and caspase-7, which are responsible for executing the final steps of apoptosis. These effector caspases cleave specific cellular substrates, including structural proteins and DNA repair enzymes, leading to cellular breakdown and programmed cell death [25].

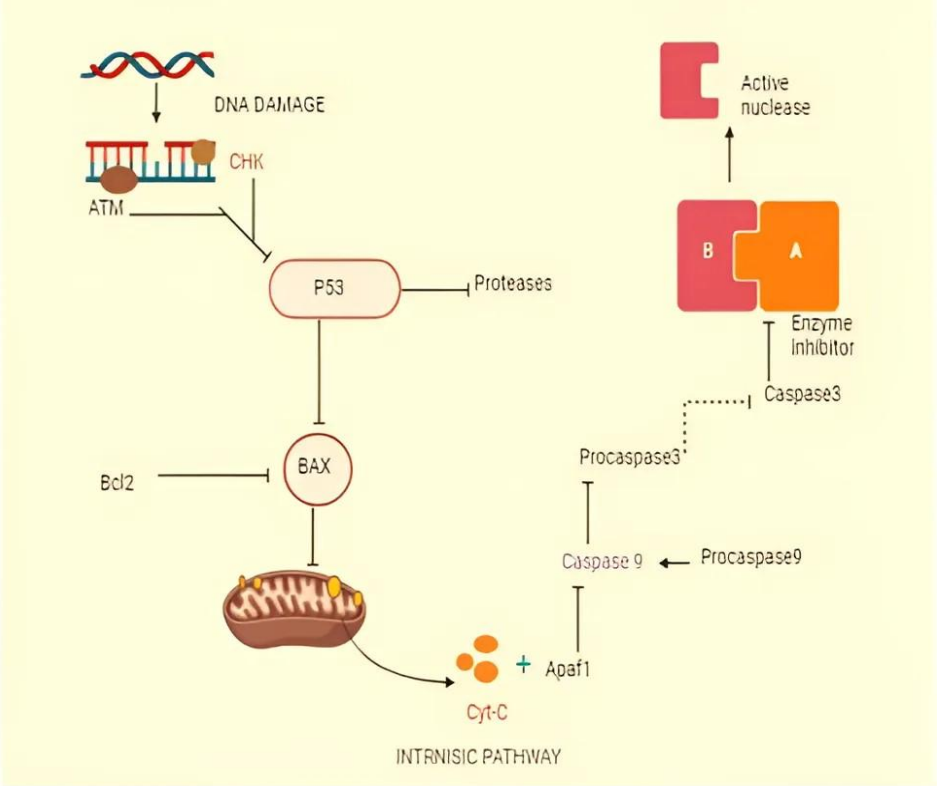

Fig. 2. Schematic diagram of Intrinsic (mitochondrial) Pathway of Apoptosis

3.2. Extrinsic Pathway

The extrinsic pathway, also known as the death receptor pathway, is a critical mechanism by which cells initiate apoptosis in response to external signals. It begins when death ligands, such as Fas ligand (FasL), bind to death receptors located on the cell surface (Fig. 3). This binding event triggers a series of molecular events that ultimately lead to cell death. Upon binding of the death ligand to its receptor, conformational changes occur in the receptor, leading to the recruitment and activation of adaptor proteins such as FADD (Fas-associated death domain). FADD then facilitates the recruitment and activation of procaspase-8 or procaspase-10 molecules to form the death-inducing signaling complex (DISC). Within the DISC, procaspases undergo autocatalytic cleavage, resulting in the activation of caspase-8 or caspase-10. These activated initiator caspases then cleave and activate downstream effector caspases, such as caspase-3, caspase-6, and caspase-7. Effector caspases are responsible for executing the final steps of apoptosis by cleaving various cellular substrates, including structural proteins, DNA repair enzymes, and inhibitors of apoptosis (IAPs). This leads to cellular breakdown and ultimately programmed cell death[26].

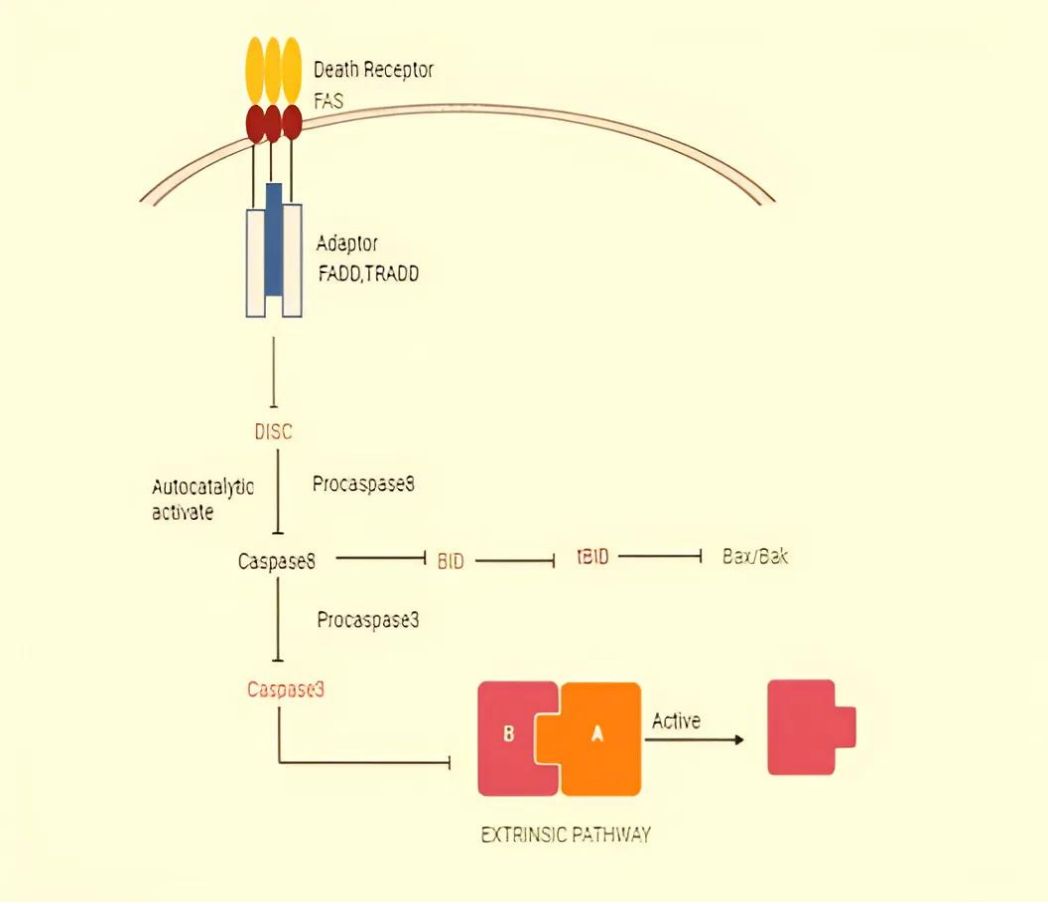

Fig. 3. Schematic diagram showing Extrinsic (death receptor) Pathway.

3.3.Granzyme B Pathway

Granzyme B-mediated apoptosis, closely linked to natural killer (NK) cells and T cells' cytotoxic activity, initiates the intrinsic apoptotic pathway in target cells. Granzyme B enters the target cell and cleaves Bid, triggering mitochondrial outer membrane permeabilization (MOMP) and the release of cytochrome c. Cytochrome c forms the apoptosome with Apaf-1 and procaspase-9, activating caspase-9 and initiating a caspase cascade, leading to cellular breakdown and apoptosis [27].

4. ROLE OF APOPTOSIS IN CANCER BIOLOGY

Apoptosis, termed programmed cell demise, serves as a critical guardian of cellular equilibrium, meticulously orchestrating the removal of aberrant cells to uphold physiological harmony. Regarding cancer development, cells obtain an enhanced potential to resist mechanisms that trigger apoptosis, marking the evasion of this process as a crucial aspect of cancer cell evolution. Cancer cells utilize a variety of mechanisms to resist programmed cell death, known as apoptosis. These processes encompass the inactivation of TSGs through genetic variations, elevated expression of anti-apoptotic proteins, activation of tumor-inducing genes, fortification of cell surviving signaling pathways, disruption of apoptotic signaling molecules, malfunctioning of apoptosis execution pathways, and viral-mediated inhibition of tumor suppressor proteins via interactions with viral proteins [28]. When apoptotic genes are hypermethylated, it often results in the suppression of apoptosis, contributing to tumorigenesis and cancer progression.

5. APOPTOTIC GENES HYPERMETHYLATED IN ORAL SQUAMOUS CELL CARCINOMA

5.1. Cadherin:

Cadherin constitutes a sizable family of cell surface proteins pivotal in processes such as cell differentiation, adhesion, and the establishment of cohesive tissue structures [29]. These proteins are categorized into three distinct types: type I, type II, and type III cadherins. Type I cadherins, encompassing epithelial (E), placental (P), neural (N), and retinal (R) cadherins, exhibit expression within the mammary gland. Type II cadherin, represented by cadherin five, assumes a critical role in preserving the structural integrity of blood vessels, alongside vascular endothelial cadherin. Cadherin 11, prevalent in osteoblasts, is indispensable for upholding the integrity of bones and joints. Type III cadherin comprises cadherin 13 and 15 [30]. Epithelial cadherin, a 120 kDa glycoprotein, encompasses three functional domains: cytoplasmic, transmembrane, and extracellular. The CDH1 gene is located on chromosome 16p22.1. This calcium-dependent adhesion molecule regulates essential physiological activities such as polarity, differentiation, and cell movement. Decreased CDH1 expression is associated

with heightened invasiveness and progression of epithelial neoplasms, including oral carcinomas. Numerous reports emphasize CDH1 expression across various cancer types. The absence of CDH1 strongly indicates potential changes in cell function and motility. In OSCC, reduced CDH1 expression is linked to an antagonistic relationship, indicating a connection between OSCC aggressiveness and decreased E-cadherin levels. The likelihood of metastasis, characterized as the “tumor avalanche” in cancerous cells, increases when mutations occur or when the gene expresses less [31]. Methylation of the CDH1 promoter is observed in around 17% to 85% of tumors found in the oral cavity.[32-34].

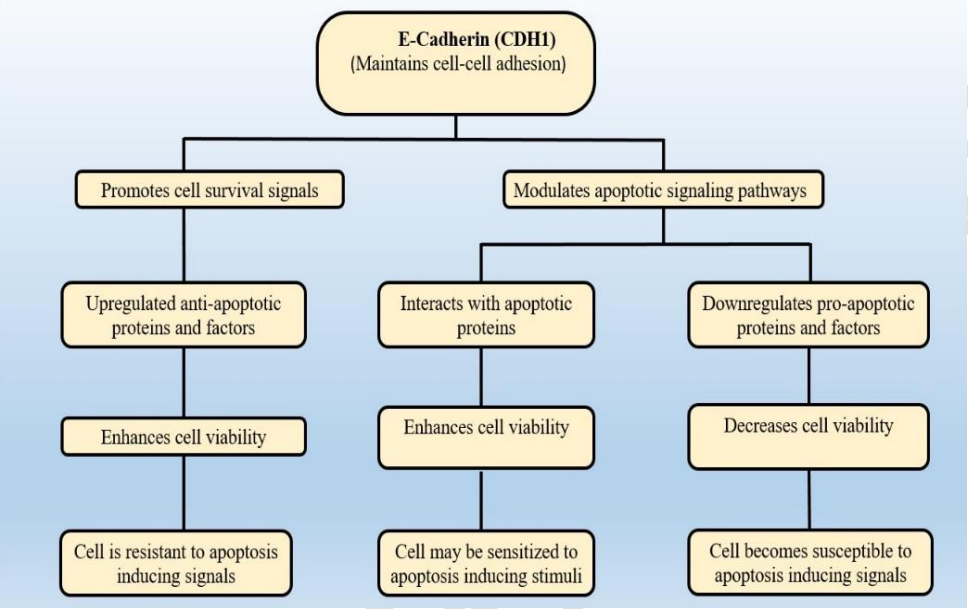

Fig. 4. Flowchart depicting the role of E-cadherin.

5.2. CASP8:

CASP8 encodes Caspase 8, also known as FLICE (FADD-like IL-1β-converting enzyme), which is a vital enzyme involved in the initiation of apoptosis. It is situated on chromosome 2q33-34 and comprises 10 exons spanning approximately 30 kilobases. There are findings indicating approximately 168 single nucleotide polymorphisms (SNPs) linked with CASP8, most of which are uncommon or nonoperative. In addition to its role in the FAS-FAS ligand-mediated extrinsic pathway, CASP8 interacts with the BH3 interacting-domain death agonist protein to modulate the intrinsic pathway, thereby engaging caspases [35-37]. Caspase-8 has been identified as a contributor to various cellular processes, including the NF-κB signaling activation, autophagy regulation, modification of endosomal trafficking, and facilitation of cell migration and adhesion. The multifaceted functions of CASP8 suggest that its impact on tumor malignancy depends on a particular cellular context, indicating that CASP8 can either enhance or suppress tumor development based on the prevailing conditions [38]. Caspase-8 becomes activated upon receiving signals from death receptors, triggered by the interaction between molecules like TRAIL or FasL and their corresponding death receptors. This activation takes place within the DISC, where Caspase-8 is inducted and undergoes autocatalytic processing, leading to its conversion into its active form. Following this, Caspase-8, once activated, commences programmed cell death by cleaving and activating subsequent effector caspases such as Caspase-3 and Caspase-7. These events result in the impactful outcome execution of apoptosis, playing a crucial role in eliminating cells with damaged DNA or those experiencing cellular stress.

5.3. Survivin:

Survivin is the unique and the smallest member within the mammalian IAP family, characterized by its distinctive structure. It consists of a sole N-terminal BIR domain (Baculovirus IAP repeat), coupled with an extended C-terminal region featuringa coiled α-helix structure [39]. The survivin protein's BIR domain is crucial for its ability to prevent apoptosis, while its coiled domain enables interaction with tubulin structures and potentially regulates cell division. Human survivin, originating from the BRIC5 gene, possesses a molecular weight of 16.5 kDa and is situated on chromosome 17 at the telomeric end, spanning 14.7 kb of genomic DNA. Within cancer cells, survivin serves two primary roles: firstly, it regulates mitosis through the assembly of the CPC (Chromosomal passenger complex) in collaboration with other proteins, and secondly, it impedes the process of apoptosis [40]. Survivin, a key player in cell survival, exerts its anti-apoptotic effects primarily by disrupting the mitochondrial-mediated apoptotic pathway. The formation of the apoptosome complex occurs in the presence of dATP involving Cytochrome c, Apaf-1, and procaspase-9, leading to the activation of procaspase-9. Survivin intervenes in this process, likely hindering caspase-9 activation by inhibiting apoptosome formation. Moreover, survivin may directly inhibit both activator caspase-9 and effector caspases-3 (Fig. 5). Additionally, survivin opposes the proapoptotic effects of Smac/DIABLO, a protein that antagonizes IAPs. By counteracting Smac/DIABLO, survivin supports the role of other IAPs, including XIAP. XIAP acts as a potent suppressor of apoptosis by directly interacting with caspases and inhibiting their function.

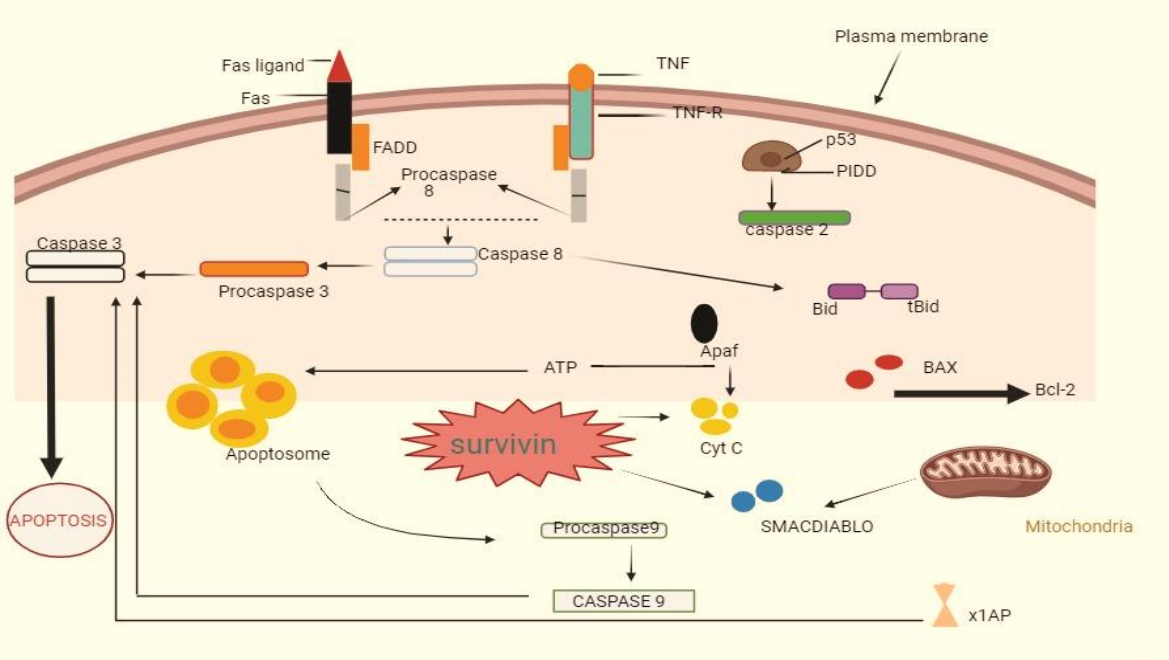

Fig. 5: Role of Survivin in the mitochondrial-mediated apoptotic pathway.

In summary, survivin orchestrates a multi-faceted strategy to inhibit crucial steps in the apoptotic pathway, thereby promoting the survival of cells and providing resistance to apoptosis mechanisms [41]. The regulation of survivin mechanisms is still not completely understood. Nonetheless, it has been noted that a variety of signaling cascades and constituents stimulate survivin in cancerous cells. Currently, it is believed that cancer cells generally exhibit more active intracellular pathways that activate survivin compared to normal cells. In contrast to cancer cell lines, variable reporter gene assays suggest a minimal survivin promoter role in normal cells, indicating differential regulation of survivin expression between these two cell types [42].

5.4. Death Associated Protein Kinase 1 (DAPK1):

DAPK1 is located at the gene locus 9q34.1. DAPK1 operates within the serine/threonine kinase pathway, regulating apoptosis through calcium/calmodulin signaling and exerting proapoptotic effects [43-45]. Its location on chromosome 9q34.1 underscores its pivotal role in maintaining cellular homeostasis by governing responses to diverse stimuli. Several studies have reported the promoter hypermethylation of DAPK1in OSCC, ranging from 18% to 27% [46-48]. This epigenetic modification hampers the expression of DAPK1, potentially impeding its role in initiating apoptosis. DAPK1's significance spans both intrinsic and extrinsic apoptotic pathways, reflecting its multifaceted involvement in orchestrating cell death processes. Its precise control over apoptosis is crucial for preventing abnormal cell survival and uncontrolled proliferation. In various cancers, particularly OSCC, the strong correlation between DAPK1 hypermethylation and the disease has been consistently demonstrated. The observed reduction in DAPK1 gene expression due to hypermethylation indicates a blockade in the apoptosis process.

5.5. p53:

The p53 gene, commonlyknown as the "guardian of the genome," serves as a pivotal tumor suppressor protein present on chromosome 17p13.1. It has a pivotal role in controlling multiple cellular functions such as cellular differentiation, cell cycle advancement, repair of DNA, and apoptosis, which is crucial for preserving cellular balance. In response to unfavorable changes, whether arising from external or internal factors, the expression of p53 is elevated, triggering a series of events that temporarily arrest cellular functions, allowing for DNA repair. Significantly, in the context of OSCC and diverse cancer types, p53 gene mutations are commonly identified. The methylation status of p53's promoter region, an epigenetic modification, falls within the range of approximately 25% to 69% [49]. This epigenetic alteration contributes to the reduced activity of p53, compromising its tumor-suppressive functions. Moreover, p53, as a critical regulator of apoptosis, exerts influence over the intrinsic (mitochondrial) apoptotic pathway. In instances of cellular stress, DNA damage, or other unfavorable conditions, p53 becomes activated.

Activated p53 has a central role in triggering the mitochondrial apoptotic pathway. This pathway entails the modulation of Bcl-2 family proteins, mitochondrial permeabilization, and the release of pro-apoptotic factors, ultimately leading to the activation of caspases and the execution of programmed cell death. A comprehensive understanding of the molecular intricacies involving p53 and the intrinsic apoptotic pathway provides insights into OSCC pathogenesis and cancer development, offering potential avenues for targeted therapeutic strategies [50-52].

5.6. RASSF1 and RASSF2:

The Ras Association domain family proteins are components of the Ras/PI3K/AKT pathways. RASSF1 is present on chromosome 3 at position 21.3, while RASSF2 is situated on chromosome 20 at position 13. Studies involving patients undergoing radiotherapy revealed that in 50% of cases, activation of the Ras/PI3K/AKT pathways occurred due to promoter methylation, leading to the silencing of the RASSF1A and RASSF2A genes [53]. This suggests that the inactivation of RASSF1A and RASSF2A through methylation-mediated gene silencing may lead to the initiation of cancer through the Ras/PI3K/AKT signaling cascade, particularly in response to radiotherapy treatment. Researchers have embarked on a promising endeavor to uncover biomarkers by discerning methylation patterns associated with tumor invasion, particularly metastasis. Methylation levels of approximately 12-38% have been detected in the RASSF1 gene, while at least one RASSF2 gene exhibits a methylation status of around 39%. This exploration into methylation patterns within the RASSF1 and RASSF2 genes represents a significant avenue for identifying potential biomarkers that could aid in understanding and monitoring tumor metastasis. Moreover, elucidating the role of methylation in these genes may offer valuable insights into the mechanisms underlying cancer progression and metastatic spread, presenting new opportunities for targeted therapies and improved patient outcomes [54].

5.7. MGMT:

MGMT also referred to as O6-methylguanine-DNA methyltransferase, is located at the gene locus 10q26.3. This enzyme plays a crucial role in DNA repair by eliminating O6-guanine-DNA adducts induced by alkylating agents. Essentially, MGMT acts as a detoxifying agent by repairing DNA damage caused by these adducts. Histone modification serves as an alternative mechanism to DNA methylation, impacting gene expression in a nuanced manner. Unlike DNA methylation, which typically leads to gene silencing, histone modification can both silence and activate gene expression. It exerts this dual influence by altering the structure of chromatin, either tightening it to suppress gene transcription or loosening it to promote transcriptional activity. This dynamic interplay between histone modification and gene expression regulation adds a layer of complexity to epigenetic regulation and contributes to the intricate control of cellular processes [55].

Table 1. List of different types of apoptotic genes hypermethylated in OSCC along with their location, function and the pathway they follow.

Gene	Location	Function	Pathway	References
Cadherin	Chromosome 16p22.1	Mediate cell-cell adhesion in epithelial tissues	Intrinsic apoptotic pathway	[56]
CASP8	Chromosome 2q33.34	Function as a tumor suppressor	Extrinsic apoptotic pathway	[57]
Survivin	Chromosome 17q25	Promote cell survival and inhibit apoptosis	Both intrinsic and extrinsic apoptotic pathways	[41]
DAPK1	Chromosome 9q34.1	Cellular processes, including apoptosis, autophagy, and cell migration	Intrinsic apoptotic pathway	[58]
p53	Chromosome 17p13.1	Regulate cell cycle progression, DNA repair, apoptosis, and senescence.	Intrinsic apoptotic pathway	[50-52]
RASSF1 and RASSF2	Chromosome 3p21.3 and Chromosome 20p13	Cellular processes, including cell cycle regulation, apoptosis, and tumor suppression.	Intrinsic and extrinsic pathways	[59]
MGMT	Chromosome 10q26.3	Repair damaged DNA caused by alkylating agents.	follows the DNA repair pathway rather than directly participating in apoptosis pathways	[60]

Apaf-1	Chromosome 12q23.1	regulate programmed cell death by facilitating the formation of the apoptosome, a large multiprotein complex.	Intrinsic apoptotic pathway	[58]

6. FUNCTIONAL CONSEQUENCES OF HYPERMETHYLATION ON APOPTOTIC GENE EXPRESSION

Hypermethylation-induced alterations in apoptotic gene expression have significant functional consequences in OSCC, impacting various aspects of tumor development, progression, and response to therapy.

6.1. Suppression of Pro-Apoptotic Genes: Hypermethylation-mediated silencing of pro-apoptotic genes such as Adenomatous Polyposis Coli (APC), Death-associated Protein Kinase 1 (DAPK1), and Phosphatase and Tensin Homolog (PTEN) in OSCC results in the suppression of apoptosis, facilitating the survival and growth of tumor cells [61].

6.2.Disruption of Apoptotic Signaling Pathways: Epigenetic alterations, including hypermethylation, can disrupt apoptotic signaling pathways by downregulating key apoptotic regulators. For instance, hypermethylation-mediated silencing of the Apoptotic Peptidase Activating Factor 1 (APAF1) gene inhibits the development of the apoptosome complex, impairing the initiation of downstream effector caspases and compromising the apoptotic response [62].

6.3. Resistance to Apoptosis Induction: Hypermethylation-mediated downregulation of apoptotic genes facilitates the development of resistance to apoptosis induction in OSCC cells. For example, hypermethylation of the CASP8 (Caspase 8) gene attenuates caspase-8 expression, thereby impairing extrinsic apoptosis signaling and rendering tumor cells less susceptible to death receptor-mediated apoptosis [63].

6.4. Enhanced Tumor Survival and Metastasis: The dysregulation of apoptotic gene expression due to hypermethylation confers a survival advantage to OSCC cells, facilitating tumor growth, invasion, and metastasis. Hypermethylation-mediated silencing of genes like RASSF1A and p73 disrupts apoptotic pathways, promoting tumor cell survival and facilitating metastatic spread.

6.5. Impact on Therapeutic Response: Epigenetic alterations in apoptotic genes also influence the response of OSCC to therapeutic interventions. Hypermethylation-induced downregulation of apoptotic genes can confer resistance to chemotherapy and radiotherapy, limiting the efficacy of these treatments and contributing to disease recurrence and poor patient outcomes.

7. THERAPEUTIC IMPLICATIONS AND FUTURE PERSPECTIVES

7.1. Targeting Hypermethylation for OSCC Treatment:

Targeting hypermethylation as a therapeutic strategy for oral squamous cell carcinoma (OSCC) holds significant promise. One example of a demethylating agent is 5-azacytidine, which has been investigated for its ability to inhibit DNA methyltransferases and promote DNA demethylation. Another agent, decitabine, also functions similarly and has shown efficacy in other cancer types. In preclinical studies, these agents have demonstrated the potential to reactivate silenced genes and enhance the effectiveness of chemotherapy and immunotherapy in models of OSCC. Additionally, targeted therapies focusing on specific hypermethylated genes such as APAF1 and PTEN are being explored. For instance, researchers are investigating small molecule inhibitors that selectively target proteins involved in gene silencing through DNA methylation or histone modification. These inhibitors offer a more targeted

approach to reversing hypermethylation and restoring gene function. Furthermore, combination therapies that include demethylating agents along with conventional chemotherapy or novel targeted agents represent a promising approach. By merging these substances, there is potential to attain synergistic results, ultimately enhancing the treatment results for individuals diagnosed with OSCC.

7.2. Epigenetic Therapy Approaches in OSCC:

Epigenetic therapeutic approaches for OSCC target the modulation of gene expression through modifications in DNA methylation, histone acetylation, and ncRNA expression. A particular approach involves employing demethylating agents like 5-azacytidine and decitabine, which hinder DNA methyltransferases and facilitate DNA demethylation. This process results in the restoration of tumor-suppressor gene expression [64]. Histone deacetylase inhibitors are a potent category of epigenetic therapy agents that have shown promise in OSCC. Drugs like vorinostat and romidepsin reverse histone hypo acetylation, resulting in the re-expression of silenced genes and induction of apoptosis in OSCC cells [65].

Emerging epigenetic targets for OSCC therapy include lysine-specific demethylase 1 (LSD1) and bromodomain and extra-terminal domain (BET) proteins. BET inhibitors disrupt chromatin structure and inhibit oncogenic transcriptional programs, showing efficacy in preclinical models of OSCC [66]. Similarly, LSD1 inhibitors reverse histone methylation patterns and suppress tumor growth in OSCC xenografts. Moreover, dysregulated microRNAs are being targeted for OSCC therapy using antagomirs or microRNA mimics. These molecules play critical roles in OSCC pathogenesis and their modulation offers another avenue for epigenetic-based therapeutic intervention [67]. These epigenetic therapy approaches hold promise for OSCC treatment by targeting key molecular mechanisms involved in tumor development and progression. Nevertheless, additional investigation and clinical trials are imperative to comprehensively grasp their effectiveness and potential adverse reactions in individuals with OSCC.

7.3. Challenges and Opportunities in Translating Research Findings into Clinical Practice:

Translating epigenetic therapies into clinical practice for OSCC faces significant challenges. A major obstacle is the absence of biomarkers predictive of treatment response, making it difficult to select patients who will benefit from these therapies and monitor their efficacy. For example, in a study by Kurokawa et al., the authors emphasized the need for biomarkers to predict the response to epigenetic therapy in OSCC patients [68]. Additionally, off-target effects and toxicity associated with epigenetic drugs, such as vorinostat and romidepsin, may limit their clinical utility [69]. Moreover, the heterogeneity of OSCC and the intricate interplay between genetic and epigenetic alterations present challenges for developing personalized treatment strategies. For example, in a review by Vered et al., the authors discussed the complexities of addressing the genetic and epigenetic heterogeneity of OSCC in clinical practice [70].

However, advancements in high-throughput sequencing technologies and bioinformatics tools offer promise for identifying novel epigenetic biomarkers and optimizing treatment regimens in OSCC patients. These tools enable researchers to analyze large datasets and identify potential biomarkers associated with treatment response and disease prognosis [71]. Furthermore, collaborative efforts between basic scientists, clinicians, and pharmaceutical companies are crucial for conducting well-designed clinical trials to validate the efficiency and safety of epigenetic therapies in OSCC. By pooling expertise and resources, these stakeholders can overcome challenges and accelerate the translation of epigenetic therapies from the bench to the bedside [72].

7.4. Future Directions and Emerging Strategies:

Future directions in epigenetic therapy for oral squamous cell carcinoma (OSCC) entail investigating combination treatments that target multiple epigenetic regulators and pathways. By merging demethylating agents, histone deacetylase inhibitors (HDACis), and targeted drugs, researchers aim to surmount resistance mechanisms and enhance treatment efficacy in OSCC patients. Several DNA methylation inhibitors have been developed, including 5-Aza-2′-deoxycytidine (5-Aza-dc). This nucleoside DNMT inhibitor (DNMTi) works by being incorporated into DNA during replication. Once integrated, it irreversibly binds to DNMT1, inhibiting its activity and leading to demethylation of DNA [73].

For example, Cheng et al. (2009) demonstrated the effectiveness of a demethylating agent combined with HDACi in inducing apoptosis in OSCC cells. Their study concluded that demethylation of the APAF-1 promoter induces apoptosis in OSCC both in vitro and in vivo. Specifically targeting the APAF-1 promoter region for demethylation restored APAF-1 expression, promoting apoptotic cell death in OSCCs. These findings suggest that APAF-1 promoter demethylation could serve as a potential therapeutic strategy for inducing apoptosis and inhibiting tumor growth in oral squamous cell carcinoma. Furthermore, there is growing interest in uncovering new epigenetic vulnerabilities and actionable targets through comprehensive omics profiling and functional genomics studies. This methodology will pave the way for the formulation of bespoke medical approaches precisely customized to the unique needs of each OSCC patient. For instance, Singh et al. highlighted the therapeutic potential of targeting lysine-specific demethylase 1 (LSD1) in OSCC.

Additionally, integrating epigenetic therapies with immunotherapy and radiotherapy holds promise for achieving lasting responses and enhancing survival rates in OSCC patients. Preclinical investigations have demonstrated that epigenetic modulation can amplify the effectiveness of immunotherapy agents, such as immune checkpoint inhibitors, in OSCC. Moreover, ongoing research focuses on developing non-invasive epigenetic biomarkers for early detection, prognosis, and monitoring treatment response in OSCC. For example, Kurokawa et al. showed the potential of demethylating the APAF-1 promoter as a biomarker for inducing apoptosis in OSCC cells. These advancements have the potential to transform clinical management strategies for OSCC, ultimately leading to improved patient outcomes.

8. CONCLUSION:

Essentially, the hypermethylation of apoptotic genes in OSCC signifies a fundamental molecular alteration that significantly impacts the progression and development of this cancer type. The atypical DNA methylation seen in OSCC interferes with the typical operation of apoptotic genes, facilitating the avoidance of programmed cell death and fostering unrestricted cellular proliferation. This disruption, especially impacting tumor suppressor genes linked to apoptosis, plays an important role in the initiation and progression of OSCC. The intricate interplay between epigenetic modifications, specifically DNA hypermethylation, and the apoptotic pathway highlights the intricate nature of OSCC. This disruption in the apoptotic process is a characteristic feature of cancer, enabling malignant cells to persist and proliferate. The identified hypermethylation patterns may extend beyond individual genes, indicating a broader epigenetic landscape that supports the survival and expansion of OSCC cells. From a clinical perspective, comprehending the significance of hypermethylation in apoptotic genes holds potential as a biomarker for diagnosing, prognosis, and responding to treatment in OSCC. Targeted therapies aimed at reinstating normal apoptotic functions could improve the efficacy of conventional treatments, opening new avenues for managing OSCC. Furthermore, environmental factors and lifestyle choices, such as tobacco and alcohol use, have been recognized as influential in shaping hypermethylation patterns in apoptotic genes. This understanding of the interplay between external factors and epigenetic modifications provides valuable insights for preventive strategies and personalized approaches to OSCC. To advance our knowledge and therapeutic approaches, future research should concentrate on identifying the specific apoptotic genes affected by hypermethylation in OSCC and deciphering the intricate regulatory networks at play. Integrating multi-omics approaches and conducting extensive clinical studies will enrich our understanding of the role of hypermethylation in OSCC.

ABBREVIATIONS:

1. OSCC- Oral squamous cell carcinoma
2. NCDs- Non-communicable diseases
3. HPV- Human papillomavirus
4. RNAi- Ribonucleic acid interference
5. DNMTs - DNA methyltransferases
6. TSG - Tumor suppressor genes
7. HDACs- Histone deacetylases
8. ncRNAs- Non-coding RNAs

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