

A review on genes contributing to the development and etiology of oral cancer

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Abstract

Oral cancer is a major public health concern worldwide. It has one of the highest incidences of cancer in the world, and despite breakthroughs in research and treatment, survival rates have remained stagnant in recent years, posing a constant challenge to biomedical sciences. In most races, men are two to three times more likely to develop oral cancer than women. The purpose of this article is to outline the key aspects of oral cancer, including clinical, histological, and molecular concepts, in order to understand its biological pathways and to enable readers and researchers to create maps that can be used for localization and integration in this growing body of knowledge. It is in the top 10 in cancer incidence, and despite advances in research and treatment, survival has not improved significantly over the past few years, which is an ongoing challenge for biomedical sciences. The purpose of this paper is to report on key aspects of this cancer, integrating clinical, histological, and molecular concepts to better understand its biological pathways and to allow readers and researchers to construct maps that can be used to place and integrate this growing amount of information.

Keywords: Oral squamous cell carcinoma, Cancer development, Cancer Biomarkers

1. INTRODUCTION

Oral cancer is a type of cancer that develops in the lips or mouth. 90 percent of malignancies in dentistry are histologically derived from squamous cells, which are traditionally referred to as oral squamous cell carcinoma (OSCC) (Kademani). It has varying levels of differentiation and a proclivity for lymph node metastasis. The proper cell mechanism of a human's complete life system depends on cell homeostasis (Hill & Artis, 2009).

Any disruption in cell homeostasis can lead to a variety of diseases. An increase in cell proliferation or a decrease in cell death disrupts the unusually well-regulated balance between cell proliferation and cell death, resulting in a neoplasm that can grow into a tumor over time (N. Lee & Kim, 2017). Tumors are classified as benign or malignant. The tumor's propensity to invade/spread surrounding normal tissue/distant body sites can cause cancer to upsurge (grow out of control) (Han et al., 2018).

Cancer is becoming problematic all around the world, and it is the second greatest cause of mortality. Cancer is anticipated to climb by 50% by 2020, according to the World Health Organization (Arnold et al., 2020). Cancer is a multistep process that involves genetic mutations/deletions as well as epigenetic alterations including DNA methylation and histone modification. Tumors with acceptable genomic abnormalities can acclimate and proliferate on their own, and advance for their survival and epigenetic variables, such as changes in DNA methylation (Raimondo et al., 2015). Gene expression changes are inherited but not caused by a change in the DNA sequence (Osborn et al., 2003).

Epigenetic alterations, according to the epigenetic hypothesis, can play a significant role in the development and progression of cancer (Wong, Lee, Ching, Jin, & Ng, 2003). Human cancer cells, in addition to various genetic mutations, have been found to exhibit worldwide epigenetic aberrations, according to recent advancements in the science of epigenetics (Ozen et al., 2013). At all phases of cancer development, genetic and epigenetic changes interact and work together to promote cancer progression (Byler et al., 2014).

Epigenetics affects cell transcription, causing gene expression to be disrupted. As a result, gene function is disrupted, and cells begin to change into cancerous tumors (Feinberg, Ohlsson, & Henikoff, 2006). DNA methylation, histone modification, and short noncoding microRNAs are the three types of epigenetic alterations that are commonly classified (miRNA). DNA methylation is the most researched of these pathways, and it is the subject of this review.

2. RISK FACTORS

Oral cancer is a preventable disease in which smoking and alcohol considered major risk factors, and are present in 90% of cases and have a synergistic effect (Pelucchi, Gallus, Garavello, Bosetti, & La Vecchia, 2006).

Tobacco

The International Agency for Research on Cancer concluded (2007) that "there is good evidence that snuff is carcinogenic, for example, it causes cancers of the mouth and pancreas". Smokers have a 3-fold risk of oral cancer as non-smokers. In addition, people who quit smoking four years ago had a 35 percent lower risk of oral cancer than those who continued to smoke, and people who had no history of smoking for more than 20 years had a lower risk of developing oral cancer than people who had never smoked. Environments with cigarette smoke are also dangerous; those who never smoke but have been exposed to an environment with cigarette smoke (involuntary

smoking) have higher risk of oral cancer than those who have never smoked but have never been exposed to cigarettes (P. N. Lee, Thornton, Forey, & Hamling, 2017).

Cigarette smoke weakens the immunity of the oral cavity by promoting gingivitis, periodontitis and oral cancer (Stämpfli & Anderson, 2009). This smoke contains several elements that promote cancer, and they can basically be divided into three distinct groups: nitrosamines, benzopyrene, and aromatic amines (Rivera, 2015). These chemicals undergo coordinated alterations in oxidase so that the final product becomes electron-deficient and becomes a reagent that covalently binds to DNA, creating regions of adduct mutations. In addition to oxidation, enzymatic or non-enzymatic metabolism also produces carcinogens, such as free radicals, which have unpaired electrons, making them extremely reactive and able to promote mutations through complex mechanisms (Phaniendra, Jestadi, & Periyasamy, 2015). Smoking snuff exposes the oral epithelium to free radicals of oxygen and nitrogen, which affect antioxidant defence mechanisms. Elevated levels of these free radicals are found in precancerous lesions of the mouth and in cancer (Choudhari, Chaudhary, Gadail, Sharma, & Tekade, 2014).

Alcohol

Ethanol can act as a local and systemic risk factor: increase the permeability of the oral mucosa, dissolve the lipid components of the epithelium, lead to epithelial atrophy and interfere with DNA synthesis and repair; and it also has genotoxic and mutagenic effects, leading to a decrease in saliva flow, affecting the liver's ability to process toxic or potentially carcinogenic compounds, and long-term use of them impairs innate and acquired immunity, leading to increased susceptibility to infections and tumors (Reidy, McHugh, & Stassen, 2011).

Other factors

Among other risk factors are human papillomavirus (mainly associated with oropharyngeal carcinoma) and ultraviolet radiation (UV) (O'Neill et al., 2011). IARC classifies human papillomavirus 16 (HPV16) as the cause of oral and pharyngeal tonsil cancer, while HPV18 may cause oral cancer (Muñoz, Castellsagué, de González, & Gissmann, 2006). HPV-associated head and neck squamous cell carcinoma (HNSCC) is most commonly found in the tonsils and base of the tongue in the oropharynx, with a prevalence of 75%; HPV-associated HNSCC is rare in non-oropharyngeal areas (Alsbeih et al., 2019). The presence of HPV is an established prognostic biomarker of a good prognosis for locally advanced oropharyngeal carcinoma (Alsbeih et al., 2019). There is evidence that HPV contributes to cancer through two virally encoded proteins: the E6 protein promotes the degradation of p53 tumor suppressor gene products; E7 promotes degradation of the tumor suppressor gene product pRb (retinoblastoma protein), resulting in a dysregulation of cell cycle control, which also leads to overexpression of cyclin-dependent kinase p16Ink4a inhibitors (Thomas, Balan, & Balaram, 2015; Yim & Park, 2005). Ultraviolet radiation, mainly UVB, has also been linked to oral cancer.

3. GENE EXPRESSION

One of the chief causes of oral cancer patients' death is metastasis (Irani, 2016). The process of dispersion of oral cancerous cells from the primary tumor to surrounding organs and other parts of the body is a sequence of sequential and interdependent events (Welch & Hurst, 2019). Some patients may die because they have tumors with aggressive invasive properties (Welch & Hurst, 2019). These aggressive cancers usually have aberrant gene expression that is associated with the expression of oncogenes and a loss of tumor suppressor genes (E. Y. Lee & Muller, 2010). Cancerous cells expressed these genes may be able to leave their tumors more readily and thus metastasize to other parts of the body.

Oral squamous cell carcinoma (OSCC) are the most common histological cancer type of the oral cavity, and it is considered to have a multifactorial etiology due to the synergy of multiple risk factors (Rivera, 2015). DNA methylation is one of many epigenetic (gene expression changes without a change in DNA sequence) phenomena in eukaryotic organisms. CpG islands, a GC-rich area in the genome, are known to contain a significant fraction of the CpG dinucleotides (Singal & Ginder, 1999). They are usually found near the 5' end of many promoter genes and are not methylated in most cases, regardless of the gene's expression state. In eukaryotes, DNA methylation comprises an enzyme activity that creates 5-methylcytosine by adding a methyl group to the carbon 5 position of the cytosine ring within the CpG islands (Moore, Le, & Fan, 2013).

The general methyl donor S-adenosyl-L-methionine is used to methylate cytosine by a family of DNA (cytosine-5) methyltransferases (DNMTs) (SAM) (Takeshita et al., 2011). Around 70-80 percent of the methylation CpG islands in the human genome are affected in somatic cells, out of a total of 1% of all methylated CpG islands in the human genome (Ziller et al., 2013). The appearance of the main groove of DNA to which the DNA binding proteins bind is often altered when methyl groups are introduced to the 5-position of cytosines in the CpG islands. These epigenetic alterations can be referred to as molecular markers because they can be duplicated after DNA synthesis and passed down to future generations.

Normal development and DNA methylation is one of the most important epigenetic alterations in the genome that affects its function (Wood & Oakey, 2006). DNA methylation has been found to be an important feature for individual cell viability in studies (Ferguson-Smith, 2011; Yoder, Walsh, & Bestor, 1997). A recessive lethal phenotype was seen in mouse embryos with homozygous mutations in the murine DNMT gene, resulting in the embryos' mortality before mid-gestation, indicating that DNA methylation is necessary for normal development (Jacks, 1996). Methylation is a crucial mechanism that has been linked to the inactivation of the X-chromosome in females (Sado, Okano, Li, & Sasaki, 2004). The genes on the inactive X-chromosome are frequently methylated, whereas those on the normal or active X-chromosome are unmethylated in their CpG islands, according to several research. This was shown in a mouse model of the P_{gk}-J gene, where 120 CpG residues on the CpG Island were found to be methylated in the inactive X chromosome but unmethylated in the active state

(Razin & Cedar, 1991). It's also been discovered that methylation patterns at fertilisation varied between maternal and paternal alleles.

Imprinted genes are a type of gene that is differently expressed in mammals and is also influenced by the allele's paternal origin. Cytosine methylation at differentially methylated regions (DMRs) regulates the expression of these genes. According to studies, CpG dinucleotides are found in parasitic DNA elements (Retrotransposons) such as L1 Elements, ALU repeats, and endogenous retroviruses. CpG islands are abundant in these elements, which make up over 40% of the human genome (Medvedeva et al., 2010). The presence of such parasitic DNA fragments within the human genome can endanger both the structural and functional features of the genome, potentially leading to chromosomal abnormalities such as translocations and gene activity disruption. As a result, DNA methylation may help to maintain complete genomes by masking or inhibiting homologous recombination between such repeats (Yaari et al., 2019).

Targeting gene inhibitors

The p53 protein functions can be impacted by genetic mutations or deregulation of its regulators (Herrero, Rojas, Misiewicz-Krzeminska, Krzeminski, & Gutiérrez, 2016). The principle p53 negative regulator is MDM2, which enhances its degradation. Mit-MDM2, MI-219, Nutlins and their later generation RITA (reactivation of p53 and induction of tumor cell apoptosis) are special molecules that block the binding of MDM2 to p53, which restores p53 tumor suppressor function (Shangary & Wang, 2009). Treatments focused on altering the pathways particularly in cancers with mutated p53, are more efficient (Hu et al., 2021). Some of these treatments are more effective for one subgroup of patients with HPV-related oropharyngeal cancer. People who are patients with HR-HPV-associated cancer that is p53 mutated. A technique called CH1 domain inhibitor (CH1i) weakened the stabilization for p53 on E6 HPV 16 protein (Brown, Kowalczyk, Taylor, Morgan, & Gaston, 2008). This allowed p53 to be more acetylated, which lead to greater upregulation of p53 transcription.

The discovery of the p53 homologs, p63 and p73, has interesting implications for cancer research (McKeon, 2004). First, multiple p63 and p73 genes are present in mammalian cells, suggesting that these homologues function as well (McKeon, 2004). Even though p63 and p73 bind to dsDNA in the same manner as p53, they seem to have some roles distinct from p53. With the same mutations as p53, p63 and p73 also cause cancer development. These homologs may prove to be novel anti-cancer targets having properties similar to p53 (McKeon, 2004). Also, p63 and p73 may play a crucial role in promoting resistance to the p53-dependent anti-cancer drugs.

Oral cancer, also known as OSCC, is a major and rapidly spreading concern in many regions of the world, as well as one of the most frequent and dangerous cancers. It is the sixth most frequent cancer in the world, with an estimated annual incidence of over 50,000 cases (Nagler, 2002). Every year, over 100,000 instances of oral cancer are reported in India.

According to a case study of cancer mortality in India in 2010, cancer of the oral cavity has the highest mortality rate among men and is the third most prevalent malignancy

among women. It was also shown that cancer is most prevalent in India's north-eastern regions. In Meghalaya, a retrospective study on the prevalence of Head and Neck Cancer in the state conducted in Shillong Civil Hospital from 2007 to 2011 revealed that Oropharyngeal and Oral cancer are the most common in this region (Shunyu & Syiemlieh, 2013).

DNA methylation is recognised to play a role in OSCCs, and several genes in oral cancer tissue have been methylated. Oral cancer is thought to be caused by global hypomethylation as well as promoter hypermethylation of particular genes, according to several studies (Rapado-González, López-Cedrún, López-López, Rodríguez-Ces, & Suárez-Cunqueiro, 2021). Hypermethylation of CpG islands, particularly in promoter areas, causes tumour suppressor genes to be silenced, whereas hypomethylation causes oncogenes to be activated (Esteller, 2005).

The methylation blueprint within the genome is hypothesised to play a role in the formation of neoplasia and tumour progression, specifically promoter specific hypermethylation and global (genome-wide) hypomethylation.

Hypermethylation in oral cancer

In the last few decades, epigenetic changes in relation to promoter CpG island hypermethylation has been one of the most active areas of cancer research. Promoter hypermethylation is a significant step in oral carcinogenesis, and several genes involved in cell cycle control (p16, p15), apoptosis (p14, DAPK, p73, and RASSF1A), Wnt signalling (APC, WIF1, RUNX3), cell-cell adhesion (E-cadherin), and DNA-repair are reported to be hypermethylated in OSCC (MGMT and hMLH1) (Mascolo et al., 2012).

DNA Methylation has an impact on the expression of tumour suppressor genes like CDKN2A and RASSF1 as well as DNA repair genes like MGMT (Taioli et al., 2009). Oral cavity cancer patients' tumour tissue has been shown to have abnormal gene hypermethylation of these genes. Furthermore, the promoter methylation of CDH1 has been investigated in relation to the survival of people with head and neck cancer (Taioli et al., 2009). In a semiquantitative analysis of the methylation status of 12 loci in 96 oral primary tumours and 13 cell lines, Ogi and co-workers discovered that 9 of the 12 loci examined (p16INK4A, p15INK4B, p14ARF, DCC, DAP kinase, MINT1, MINT2, MINT27, and MINT31) had aberrant methylation at various frequencies, while 3 (hMLH1, HRK, and CACNA1G) showed no methylation (Mascolo et al., 2012; Ogi et al., 2002).

p16INK4A is an early and lasting epigenetic change in oral cancer risk individuals without any visible lesions that would have indicated DNA damage in oral mucosa cells, according to PCR-single-strand conformation polymorphism (PCR-SSCP), methylation-specific PCR (MSP), and cycle sequencing. Hypermethylation of DAP kinase and MINT1's 5 CpG island was also observed to be associated with gene expression loss (Ogi et al., 2002).

Methylation profiling of genes in patients with head and neck cancer revealed a link between hypermethylation of the promoter region of DAPK1 and ADAM23 and the development of head and neck cancer, while methylation of MINT1 and MINT31 is

linked to poor oral cancer prognoses found as a senescence regulator in human keratinocytes in vitro, and aberrant methylation within 14-3-3 has been linked to reduced or absent expression at both the mRNA and protein levels in patients with squamous cell carcinomas and oral dysplasia (Ogi et al., 2002; Roberts, 2010).

RASSF2 epigenetic inactivation has a major role in OSCC, according to COBRA research, and could be exploited as a molecular target for OSCC diagnosis and treatment (Imai et al., 2008).

Methylation of RASSF1A/RASSF2A is the most common mechanism in oral cancer, according to a Taiwanese research of oral squamous cell carcinomas (Huang et al., 2009). MSP of the soluble frizzled receptor protein (SFRP1, SFRP2, SFRP4, and SFRP5) suggests that epigenetic alterations of the SFRP genes are common in HNSCC, that clonal selection for these alterations is complex, and that these alterations may be linked to carcinogenic exposures, which are known risk factors for this disease. IKK (InB kinase) plays an important role in the development of the skin and the establishment of keratinocyte phenotypes, and studies have shown that promoter hypermethylation down-regulate expression of IKK in oral carcinomas, implying that epigenetic loss of expression is closely linked with disease progression and an unfavourable prognosis (Xia, 2007).

Pyrosequencing was used to determine the methylation status of P16, RARb, E-cadherin, cyclin A1, and cytoglobin promoters in OSCC patients, and the results showed that promoter methylation of P16, RARb, E-cadherin, cyclin A1, and cytoglobin was significant in oral cancer patients (R. J. Shaw et al., 2006). EDNRB methylation in salivary rinses was independently related with histologic diagnosis of pre-malignancy and malignancy, and may have potential in classifying patients at risk for oral pre-malignant and malignant lesions, according to PCR (Q-MSP) analysis of salivary rinses of patients with oral lesions on the methylation status of EDNRB and KIF1A (Pattani et al., 2010).

Furthermore, COBRA study of methylation status in oral cancer tissues revealed that the LRP1B (low density lipoprotein receptorrelated protein 1B) promoter is frequently methylated in primary OSCC (Nakagawa et al., 2006). MTNR1A was found to be the most likely target for epigenetic silencing and to play a major role during oral carcinogenesis in an array-CGH investigation and analysis of OSCC cell lines and tumour tissues (Nakamura et al., 2008).

Hypomethylation in oral cancer

Shaw (2006) stated in his research that the overall pattern of hypomethylation in human cancer has gotten less attention than hypermethylation in specific sections of the genome (R. Shaw, 2006).

Global genomic hypomethylation in malignant tissue is a frequent hallmark of a wide range of malignancies as well as neoplasia, ranging from solid tumours including breast, colon, oral, and lung cancers to cancers of the blood (Bhatt, Mathur, Farooque, Verma, & Dwarakanath, 2010).

Oncogene activation is mostly linked to hypomethylation. It promotes carcinogenesis

by causing genomic instability, which can result in the creation of aberrant chromosomal structures and the activation of repetitive DNA elements that are normally silent (Pfeifer, 2018). People with chronic periodontitis, regardless of smoking history, had a larger percentage of hypomethylation of the IL8 gene than controls in epithelial oral cells, according to methylation specific PCR of genomic DNA from epithelial oral cells (Oliveira et al., 2009).

Because hypomethylation of promoter regions of certain genes promotes target gene production and promoter hypomethylation is linked to global methylation levels, evidence suggests that this alteration could activate or enhance oncogene activation (Hur et al., 2014).

Histological examinations revealed that DNA hypomethylation suppressed the formation of squamous cell carcinomas in a preferential manner (Baba et al., 2009). This epigenetic pharmacological change could be effective in the prevention and treatment of malignancies of the upper digestive tract.

Depending on the type of molecules and the condition in question, the term "biomarker" or "biological marker" has been defined by a number of researchers. In terms of clinical research, Strimbu and Tavel (2010) defined biomarker as "generalised medical symptoms that can be quantified properly and reproducibly and whose goal is to signal the patient's external medical state" (Strimbu & Tavel, 2010).

Biomarker discovery has led to the development of a number of applications in the field of health research, including their use as a diagnostic tool for identifying disease conditions, a tool for disease staging, an indicator for disease prognosis, and a tool for predicting and monitoring clinical response to an intervention (Biomarkers Definitions Working Group, 2001).

Identification and creation of possible biomarkers has become a crucial tool in cancer research for disease genesis, progression, treatment, and monitoring (Macklin, Khan, & Kislinger, 2020). Cancer biomarkers include genetic mutations (genome/mitochondrial genome), epigenetic alterations (DNA methylation/histone modifications), quantitative and qualitative changes in mRNA expression, protein synthesis, and serum antibodies.

4. DISCUSSION

Oral cancer is a diverse, aggressive, and complicated disease. The most common forms of treatment include surgery, chemotherapy, radiotherapy, and immunotherapy either alone or in combination. Despite therapy advancements in this and many other cancers, the prognosis for individuals with OSCC is still dismal. Every form of treatment has its own setbacks. Surgery results in a considerable loss of oral capabilities, which is followed by numerous remedial procedures that result in significant deformity and a protracted road to recovery. The best way to increase patient survival is still early diagnosis and treatment.

Alternative therapies

In order to compensate for defective genes or produce a useful protein, gene therapy involves inserting genetic material into cancer cells (Das et al., 2015). In order to convey a gene, viruses are genetically modified into vectors, however viruses by themselves do not cause disease. Due to its unusual preference for cells lining the upper aerodigestive tract, adenovirus is the most often used vector in p53 gene therapy for oral malignancies. Clinical studies based on injecting modified p53 adenoviruses (Ad-p53) have demonstrated that it is an effective treatment that can cause apoptosis and make oral cancer cells more susceptible to chemotherapy and radiation (Brown et al., 2008; Das et al., 2015; Hu et al., 2021; McKeon, 2004).

Even though chemotherapy using drugs like cisplatin, carboplatin, 5-fluorouracil, paclitaxel, and docetaxel is included among the approaches to treating OSCC, such as surgery, radiation therapy (external beam radiotherapy and/or brachytherapy), and coadjuvant therapy, it still has a high financial cost and is one of the most harmful treatments available.

Usually, one or a combination of these options is used to treat the OSCC. The decision between the two is influenced by the patient's comorbid conditions, nutritional status, ability to tolerate treatment, and desire to undergo therapy, in addition to the location, size, and stage of the primary tumour. Surgery is superior to all alternative therapy for tumours that can be removed. OSCC patients are often diagnosed in stage I or stage II of the disease. Surgery, radiation therapy, or a combination of the two are all included in local/regional therapy. With cure rates of 80% (stage I) and 65% (stage II), these individuals have a decent outlook. Unfortunately, the majority of OSCC patients are found after the disease is already advanced (stages III or IV), with a survival rate at 5 years of less than 50% and a cure rate of 30%. Untreated patients with metastatic illness have a survival time of roughly 4 months (Berdasco & Esteller, 2019; Das et al., 2015; Geum et al., 2013; Suresh, Koppad, Prakash, Sabitha, & Dhara, 2019).

The predominant symptom of oral cancer is pain, which also significantly impairs speaking, swallowing, and chewing function. A thick trigeminal innervation, ongoing oral function, opioid tolerance, mediators in the tumour microenvironment, and lack of palliative medication are some of the factors that might induce the start or worsening of pain.

5. CONCLUSION:

The goal of this review was to focus on oral cancer and to provide mechanisms that alters gene expression in mouth cancer, as well as the fact that such changes could be used as potential biomarkers in cancer diagnoses and disease monitoring. By combining a whole genome investigation with a candidate gene strategy, we hope to find "new" and "novel" epigenetically controlled target genes that contribute to the development and genesis of oral cancer.

Conflict of interest:

The authors declare no conflict of interest.

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