Promoter Hypermethylation of Apoptotic genes in Oral Squamous Cell Carcinoma

Neha Pal1, Shalini Rathore1, Priya Kaushik1, Swati Tyagi1, Anshuman Kumar2 and Abhimanyu Kumar Jha1*

1Department of Biotechnology, Faculty of Life Sciences, Institute of Applied Medicines and Research, Ghaziabad, Uttar Pradesh, India
2Director, Surgical Oncology, Dharamshila Narayana Super speciality Hospital, Delhi, India

Abstract

Cancer is one of those diseases whose treatment requires more rapid and potent response and epigenetic modifications have emerged one amongst the cancer hallmarks, commutating the concept of malignant pathologies as being singly genetic-based conditions. Oral Cancer is the type of malignancy that is found in any area of mouth and nearby regions. The epigenetic landscape is accountable for traditional development however additionally for the non uniformity among tissues in terms of gene expression patterns. Dysregulation in these mechanisms has been related to illness stage, and raised attention is currently granted to cancer so as to require advantage of those modifications in terms of novel therapeutic methods or diagnosis/prognosis tools. Epigenetics has revealed a diagnostic tool that is tumour markers which is also known as biomarkers. These biomarkers are either methylated at their CpG islands called as DNA hypermethylation or another modification could be possibly observed that would help us to identify the neoplasm for its medication because it is usually symptomless at its beginning stages. This review encompasses the different apoptotic genes that have been found as a biomarker for diagnosing the OSCC (Oral Squamous Cell Carcinoma) and how they are related to epigenetics. This can hold an immense importance in the treatment of cancer and could help in discovering novel therapeutic ways in its treatment.

Keywords: Oral Cancer; Epigenetics; DNA Hypermethylation

Introduction

Cancer stands among the foremost advanced human diseases with multiple levels of regulation. Despite the numerous progress that has been created in basic and clinical analysis, the incidence associated mortality rates related to malignant pathologies are still at high levels with an expected raised rate within the next fifteen years Irimie et al. [1]. The sixth most typical malignancy that has been found is Head and Neck cancer, wherever carcinoma represents the foremost frequent subtype inside this spectrum Irimie et al. [2], Hema et al. [3]. Also, Oral Squamous Cell Carcinoma (OSCC) accounts for roughly ninetieth of the oral subtype, being far and away the foremost common sort of malignancy inside the mouth Parkin et al. [4], Stewart et al. [5]. Apoptosis is that one pathway that could lead the elimination of oncogenic cells. Apoptosis can be explained as a process in which natural death of cells is initiated to maintain the cellular homeostasis. A variety of changes are done in the structure of cells by apoptotic proteins that eliminate the cell out of body. In this mechanism events occurs which result into formation of cascades that encounters the already anteceding cells leading them to demise, also known as “programmed cell death”, Elmore [6], Hervouet et al. [7]. The initiation and maturation of oral carcinoma caused by total of genetic changes combined with environmental risk factors (special mention to alcohol and tobacco use however various viral infectious agents and persistent inflammations) cause changes within the pursuit of cancer causing genes i.e., oncogenes and tumour suppressor genes Perez et al. [8], Irimie et al. [9].

The extent of complexity behind the unusual expression and performance of these genes is extraordinarily high, wherever epigenetic mechanisms are standing as another section of regulation besides the changes within the DNA sequence, that embrace mutations, deletions, and amplifications Lingen et al. [10]. But it has been observed that this mechanism suffers with problems or gets defected in various human diseases such as in cancer. As cancer is one of the major concerns of today’s world and it has been observed that the uncontrolled growth of cells resulting tumour escapes the apoptosis process. The various genes that plays role in apoptosis are silenced by a process named DNA hypermethylation. There are also various other epigenetic modifications that have their crucial role in oral cancer such as DNA hypomethylation, histone modification, micro RNA’s and many other.
Apoptosis

Apoptosis is a highly organized process that involves a list of events for eliminating the cell out of body. The major two different types of mechanisms are as following:

i. Extrinsic pathway or death receptor pathway

ii. Intrinsic pathway or mitochondrial pathway

iii. Additional pathway - Granzymes / perforins pathway

The procedure begins by triggering the caspase-3 followed by DNA fragmentation, degradation of cytoskeletal and nuclear proteins, formation of apoptotic bodies, expression of ligands for phagocytic cell receptors and finally uptake by phagocytic cells in extrinsic, intrinsic and granzyme B pathways. Grainzyme pathway does not depend upon degradation of capsase as it initiates parallel Martinvalet et al. [11], deflections in apoptotic process has been observed due to tumourgenesis (cancer) due to which various pursuits can’t carried out properly as deactivation of propapoptotic genes and translation of antiapoptotic genes occurs in high quantity promoting uncontrollable growth of oncologic cells Evan et al. [12] (Figure 1).

Oral Cancer

Oral cancer is a form of cancer that is found in the middle of vermilion border of lips and the junction of the hard and soft palates or the posterior of the tongue. It is a type of major malignancy found over the world. Although it was reported that oral squamous cell carcinoma (OSCC) is the most found subclassified malignancy within the oral cancer. The major causes of oral cancer can be either alcohol consumption or tobacco use or it could be atrocious inflammation in the oral mucousal cavity, there have been findings that suggests that global hypomethylation is connected to smoking Datta et al. [13], Esteller [14], Blot et al. [15] while drinking has been associated with CpG hypermethylation according to clinical studies effected on patient samples and also in murine models for oral malignancies. It has been estimated that globally the rate of death due to oral squamous cell carcinoma (OSCC) is more than 1,000,000 and 20 per 100,000 people of India are over blown by oral cancer which indicates about 30% of all types of cancer Sankaranarayanan et al. [16], Parkin et al. [17]. According to the Globocan (2018), 572,034 (3.2%) new cases for oesophageal are found out of 18,078,957 i.e. total 508,585 (5.3%) deaths in 2018 out of total 9,555,027.

Correlation between Oral cancer and Wnt Signalling Pathway

Wnt-1 was identified as proto-oncogene protein present on gene locus 12q13.12. Earlier mouse mammary tumour virus (MMTV) triggered the mammary oncogene which was then appeared as Wnt-1. The decline in pursuit of GSK-3 β and rise in β catenin pool, regulating cadherin catenin complex creation and cell-to-cell adhesion, and aiding cells from apoptosis by operating tyrosine kinase receptors Brown et al. [18]. Due to the rise in transcription elements from head and neck OSCC line, the Wnt1 genes participate sometimes. The discovery of anti-Wnt1 antibodies declined the cyclin D1 and β-catenin proteins formation in OSCC line by decreasing the Wnt1/frizzled-dependent transcription elements (TCF/LEF) Muzio et al. [19], Rhee et al. [20]. Initiation of apoptosis occurs due to choked Wnt-1 signalling. The mechanism which involves adherence molecules, elements participating in transcription and efficacious oncogenes helps to decipher the relation between oral cancer and Wnt1 signalling pathway.

Epigenetics

Epigenetics can be explained as changes in chromosome and genes that affects gene activity and expression. Epigenetics in oral cancer has been proving as a revolutionary step in diagnosis and treatment of cancer. It includes DNA methylation, histone modification and post-transcriptional gene down-regulation by microRNAs Santoro et al. [21]. These changes are not like genetic changes; they alter the DNA and histone by various mechanisms. Out of all these DNA methylations have been a hot topic for scientists in OSCC, which could suitably prove as a biomarker for the tumour detection.
**DNA Methylation**

In this process addition of methyl groups are done on specific sites known as CpG islands that contain plenty of CpG. This could be done by using enzyme DNA Methyltransferase. Several forms of DNMT’s are present such as DNMT1, DNMT2, and DNMT3. The methyl group is transferred to the carbon on 5th position of cytosine in CpG motifs which silences the gene transcription. DNA Methylation is majorly of two types - one is DNA hypermethylation that is performed by DNMT 1, DNMT 3a and DNMT 3b. Another is global DNA hypomethylation. DNA hypomethylation has been enlisted in promoting the growth of cancer cells by various mode of actions like generation of chromosomal instability, resuscitation of transposable elements, and dissipation of imprinting Esteller [22].

**Histone Modification**

Histone proteins are susceptible to totally different modifications that include ubiquitylation, sumoylation, methylation, acylation and additionally phosphorylation. These modifications occur majorly at the N-terminal tails, influencing gene transcription and crucial signalling pathways Kouzarides [23]. Histone modification is alternate to DNA methylation as it can explicitly not only mute the gene expression, but it also stimulates the transcription.

**Hypermethylated Apoptotic Genes**

**CDH1**

CDH1 which is also known as E-cadherin is found on chromosome 16q22.1 and supports cell–cell adhesion by forming adherent junction in epithelial cells for initiating and preserving intercellular adhesion, cell polarity, intercellular signalling and structure of tissue. There have many reports that emphasize on a fact that CDH1 is being expressed in several types of cancer. And if the CDH1 is not present, it is adamantly indicating that there might be changes in cell’s function and motility. It was also observed that antagonistic gesture of OSCC has its connection with the lesser expression of CDH1 (E-cadherin). It was identified that the chances of tumour avalanche which is a known property of cancerous cells “metastasis” could happen if mutation occurs or if gene expresses less. Promoter methylation of CDH1 is found to be 17–85% in oral-cavity tumours Supic et al. [24], Chang et al. [25], Kordi et al. [26], Shaw et al. [27] (Table 1).

| Gene       | Locus   |
|------------|---------|
| CDH1       | 16q22.1 |
| MGMT       | 10q26.3 |
| SARP2      | 8p11.21 |
| Survivin   | 17q25   |
| DAPK1      | 9q34.1  |
| p53        | 17q13.1 |
| XAF1       | 17p13.1 |
| APAF1      | 12q22-23|
| RASSF1 and RASSF2 | 3p21.3/20p13 |
| p73        | 1p36.33 |
| TWIST1     | 7p21.1  |
| EDNRB      | 13q22.3 |

**MGMT**

MGMT stands for O6 - methylguanine- DNA-methyltransferase; SARP2: Secreted apoptosis-related macromolecule 2; DAPK1: Death associated protein kinase 1; p53 (Tp53): Tumor suppressor protein having 53 kiloDalton weight; XAF1: XIAP ( X-linked inhibitor of apoptosis protein ) associated factor 1; APAF1: Apoptotic protease activating factor 1; RASSF1 and RASSF2: Ras association domain family proteins; p73: Tumor suppressor protein having 73 Kilodalton weight; TWIST1: Twist- related protein 1; EDNRB- Endothelin Receptor Type B.

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**Table 1**: List of various hypermethylated apoptotic genes in OSCC.

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Wnt signalling pathway and inactivation of this factor could aid cancer development and progression. SARP proteins, additionally named sFRP (for Secreted Frizzled-Related Protein), exert differential effects on osteoblastic differentiation of mouse mesenchymal cells and cellular programmed cell death of mouse osteoblasts in vitro Cho et al. [29]. SARP2 methylation was determined in each benign and malignant tumour and no applied mathematics link can be found between SARP2 methylation and illness progression.

Survivin

Survivin is another important factor that modifies apoptotic processes in oral cancer and in other different types of cancer found in our body by modulating cell cycle, gratifying the overpowering of checkpoints. It has been recorded that the transcription of this gene rises in various tumour samples like in those that have been taken from the patients suffering from oral cancer and it has been found that it was also connected with clinical as well as pathological features; in addition this gene’s expression is absent in normal tissue Muzio et al. [30], Khan et al. [31]. Single nucleotide polymorphism was also correlated with the increased expression of survivin gene in the basic cancer research. Although there have been facts that suggest that hypomethylation Gasche et al. [32], Chen et al. [33], Hsue et al. [34] is found in this gene contradictory to normal samples where hypermethylation averts the overexpression.

DAPK1

DAPK1 stands for death associated protein kinase 1 involved in OSCC as one of the hypermethylated apoptotic gene that could serve as a biomarker in future. The location is found on gene locus 9q34.1. It is responsible for operating serine/threonine kinase pathway that initiates apoptosis by calcium/calmodulin (i.e. proapoptotic) Kulkarni et al. [35], Kato et al. [36], Ha et al. [37]. It was found from few reports that 18-27% of promoter hypermethylation was observed in DAP Hasegawa et al. [38], Sanchez et al. [39], Rosas et al. [40]. Both Intrinsic and Extrinsic type of apoptosis can be performed by DAPK. In various cancers the hypermethylation of DAPK purely indicates its correlation with disease. Methylation is responsible for the reduced gene expression of DAPK gene and the blocked apoptosis process indicates that the initiation of tumour begins and other events at molecular level also described Mittag et al. [41].

P53

P53 (TP53) is a tumour suppressor protein located on 17p13.1. This gene plays a very vital role in several processes such as cellular differentiation, cell cycle progression, DNA repair and apoptosis. If any unfavourable changes occur either due to any external or internal factors it results into rise of p53 expression that ultimately chokes cellular functions, permitting DNA repair. It has been observed that in most types of cancers, the mutation in p53 is found involving OSCC. The methylation status of p53 is approx. 25% to 69% Levine et al. [42], Boyle et al. [43], Caamano et al. [44], Baral [45]. Epigenetic changes also led the reduced activity of p53. Therefore, epigenetic silencing of p53 protein (non mutated) due to HPV virus, mainly involving HPV 16 and 18 found in OSCC like in few laryngeal cancers.

RASSF1 and RASSF2

The Ras Association domain family proteins included in the Ras/P13K/AKT pathways. RASSF1 is located on 3p21.3 and RASSF2 is located on 20p13. It was displayed by radiotherapy treatment in patients (50%) that Ras/P13K/AKT mechanisms were triggered due to promoter methylation related to RASSF1A/RASSF2A gene silencing Huang et al. [46]. It has been a promising challenge for researcher’s that could help in discovery of biomarkers by identifying the methylation patterns in the tumour invasion (metastasis). 12-38% of methylation is found in RASSF1 and methylation status of RASSF2 is around 39% in no less than one RASSF2 gene Supic et al. [24], Taioli et al. [47], Huang et al. [46].

Conclusion

These studies have emerged as an effective tool for diagnosis and prognosis of such neoplasms. DNA Methylation involves a novel marker i.e. tumour marker (biomarker) [48-65]. There have been many genes that have been investigated as a biomarker few of which are described in this review. The methylation prevents the cancerous cells from apoptosis that causes invasion of these cells due to metastasis property possessed by cancerous cells. Therefore, if possibly reversing of such epigenetic modifications occurs promoting them to apoptosis then it would also help us to treat the cancer. These biomarkers are apoptotic proteins (genes) which get inactive due to methylation. In future these new therapies if adapted to treat patients by merging this present remedy seems to be more approachable and reactivation of apoptotic mechanism then would prove as very useful in the treatment of cancer.

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