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Epigenetic Changes in Carcinomas derived from Oral Mucosa: A Review

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Abstract

Molecular cancer research studies on epigenetic changes focus on areas such as DNA methylation, histone modification, miRNA and loss of imprinting. The epigenetic changes are related to molecular biological responses, such as: cellular differentiation, development, proliferation, apoptosis, tumour suppressor or genomic instability. Most of the molecular studies on oral squamous cell carcinomas emphasized that P16, p15, p14, p53, p73 are hypermethylated. Additionally, histone deacetylase modification showed significant relation in oral squamous cell carcinoma tissues, and provided great scope on policy making areas in drug targeted therapy. miRNA deregulation such as miR-137,193a,133a,222 are positively correlated with oral squamous cell carcinoma samples. The present article reviews the epigenetic changes and its role in cancer, specifically oral squamous cell carcinoma.

Key Words

Epigenetics, DNA methylation, histone modification, miRNA, loss of imprinting.

Introduction

Oral squamous cell carcinoma is the most common cancer arising from oral mucosa, and it is the fifth most common cancer among all cancer types in general. [1] The process of carcinogenesis is a result of homeostatic imbalance between cell proliferation and cell death, specifically due to the burden of genetic and epigenetic changes at molecular level. The major aetiogenic factors of oral cancer are smoking, alcohol intake, chronic irritation (sharp teeth/restoration or denture that traumatize the oral mucosa) or association with persistent high risk Human Papilloma Virus (HPV) infection. The molecular mechanism that promotes malignant transformation of a normal appearing cell are: self-sufficiency in growth signals that allows the malignant cell to proliferate in the absence of external stimulation, insensitivity to growth inhibitory signals, evasion of apoptosis or cell death mechanism which is usually through activation of anti-apoptotic genes, unlimited replication potential of malignant cell, sustained angiogenetic mechanism, ability to invade the tissue and metastasize to distant location and defects in DNA repair mechanism leading to genomic instability and mutation of protooncogenes. [2]

Current evidences from cancer studies suggest that there is greater heterogeneity in the patterns of genetic alterations, epigenetic changes and even within homogeneous histological group.[3] Heritable changes in gene expression and chromatin structure that are not coded in the DNA sequence are termed as epigenetic changes and are considered to have a key role in the mechanism of cancer development. The epigenetic changes are gaining attention in the current cancer research strategies, due to two reasons: (1) epigenetic changes occur more frequently than gene mutation and (2) may persist for the entire life and even continue for multiple generations. [4]

Research has proven that carcinogenesis is a result of multistep mechanism through which a cell can progress into malignancy. Accumulation and functional cooperation between genetic alterations and epigenetic changes is the most important step in transformation of normal cell to malignancy. [5] Research studies that focus on role of epigenetic changes in the normal cellular processes shed a light into the carcinogenesis process. Epigenetic studies are adjunct research area in cancer studies that focus on molecular cancer research studies, example: research that aimed to understand the molecular biology of cancer tissues that are under risk for malignancy after their exposure to carcinogen. In this review manuscript, we discuss various areas of epigenetic changes in cancer, specifically epithelial carcinomas that are derived oral mucosa.

Research Methodology

The literature was searched from PubMed/Medline from 1980 t0 2017. The literature search was made using MESH terms (Medical Subject Heading) "Epigenetics" "Oral cancer" "Oral squamous cell carcinoma". A total of one hundred and thirty eight articles were exanined in the search. The first and second authors of the current paper screened the title and abstracts of retrieved studies. The full texts of suitable papers that are related to the present paper objective(s) were included. The articles that did not provide full text information, non-English papers, duplicating titles were excluded. A total of twenty six papers were selected and meaningfully summarized by all the authors for the synthesis of this general review paper.

Epigenetic Dysregulation in Cancer

Epigenetics refers to changes in gene expression and does not involve the changes to the underlying DNA sequence. Epigenetic changes are normal and regular physiological pathways that is responsible for cellular processes. [6] Various epigenetic mechanisms play a crucial role in initiation and maintenance of cellular differentiation. Age, environment, life style, diet, social and cultural practices can influence the epigenetic processes. The four major epigenetic mechanisms are DNA methylation, histone alteration, RNA-mediated modifications and loss of imprinting. The fore-mentioned mechanism is conscientious for constant dissemination of genetic activity from one generation of cells to the next during the differentiation process. [7] Any disruption in these mechanisms may lead to inappropriate genetic expression and step into carcinogenesis.

DNA Methylation:

DNA methylation refers to the covalent addition of a methyl group to the 5-carbon (C^5) position of cytosine bases that are located 5' to a guanosine base in a CpG dinucleotide. The process is mediated by different DNA methyltransferases(DNMT) enzymes, among which DNMT1 is the principal enzyme for post replication

Table 1: Influence of Epigenetic changes in cellular mechanism and possible biological consequences

No.	Epigenetic changes	Sub types in epigenetic changes	recognized mechanism in cell	Biological consequences
1	DNA Methylation	DNA hypermethylation	Activation of promoter gene	Increased proliferation
			Silencing tumour suppressor gene	genomic instability
		DNA hypomethylation	Activation of oncogene, protooncogenes	Increased proliferation
			Activation of transposable genes	Genomic and chromosomal instability
2	Histone alteration	Histone acetylation	Gain of function	Activation of tumour promoting genes
			Loss of function	Defect in DNA repair and checkpoints
		Histone methylation	Loss of cellular memory	Genomic instability and influence the cancer initiation by self-sufficiency of growth promoting signal
		Histone phosphorylation	Chromatin remodelling, intracellular signalling and transcription	Genomic instability and enhanced cellular proliferation
		Histone ubiquitination	activate transcription process	Enhanced cellular proliferation
			or inhibit transcription process	Genomic instability
3	RNA mediated modification	miRNA amplification	Activation of oncogenes	Increased proliferation and influence in cancer development
		miRNA deletion	Activation of tumour suppressor genes	Genomic and chromosomal instability and influence in the cancer initiation and cancer development
4	Loss of imprinting		Loss of imprinting mechanism	Abnormal bi-allelic expression and leads into syndromes
				Genomic instability and influence in cancer initiation

(maintenance) methylation. DNMT3A and 3B are responsible for new CpG site methylation (denovo methylation). [8]

Among the three major epigenetic mechanisms, DNA methylation plays a pivotal role in cellular differentiation processes including gene expression, gene silencing of transposable elements and defence again viral sequences. It is the most studied epigenetic mechanism in physiological and cancer researches. The two predominant types of DNA methylation are DNA hypermethylation and DNA hypomethylation.

The recognized mechanism in DNA hypermethylation is hypermethylation of CpG islands within gene promoter region leading to silencing of tumour suppressor genes and contributing in the progression of malignant process.[9] The resultant progression of normal cell into a malignant one is achieved by increased proliferation rate, growth sufficiently signals, chromosomal and genomic

instability events. The recognized mechanism in DNA hypomethylation is activation of cellular oncogenes and protooncogenes, and activation of transposable elements leading to increased mutation rate and contributing to early molecular cascade of carcinogenesis.

Activation of proto-oncogenes is associated with increased proliferation, enhanced growth sufficiency signals, transcriptional discrepancies, and genomic instability. [10]

Histone Alteration:

Histone protein plays a role in transcription of genetic information that is encoded in DNA. Histones have a structural role in chromatin architecture, and constitutes in transcription process. Acetylation, methylation, phosphorylation, and ubiquitination are the four major histone modifications and formulate a "histone code" which finally modulates the genetic code.[11] The histone modification is responsible at many cellular processes such as gene transcription, DNA repair, DNA recombination and DNA replication. Lysine residues in N-terminal tail from the histone core of the nucleosome are acetylated or deacetylated by the process of histone acetylation or deacetylation. Histone acetylation is one of the part of gene regulation process and is characteristically catalyzed by enzymes such as histone acetyltransferase (HAT) or histone deacetylase (HDAC). In the process of acetylation, a single acetyl group is transferred from one molecule to the other, whereas deacetylation is simply the reverse reaction of the above stated, and in which acetyl group is removed from a molecule.[12] The possible mechanisms in histone acetylation responsible in carcinogenesis are gain-of-function and/or loss-of-function. Histone acetylation that are involved in chromosomal translocation and result in fusion protein exhibit 'gain-of-function' by altered histone acetylation activity. Consequence of gain of function is activation of tumour promoting genes and

Table 2: Epigenetic changes in oral squamous cell carcinoma tissues and possible biological consequence and related genes or proteins

Epigenetic changes	Cellular functions	Genes or proteins that are related to cellular functions
DNA hypermethyation	Cell cycle control	P16,p15
	Apoptosis	p14,DAPK, p73, and RASSFIA
	Cellular proliferation and adhesion	APC, WIF1, RUNX3
	Cellular adhesion	E-cadherin, EDNRB
	DNA repair	MGMT, hMLH1
	Tumour suppressor	PTEN, ATM, C/EBPa,HIN1, Rb
	Epithelial mesenchymal interactions	TCF21, TIMP3, THBS1
DysregulatedmiRNA	Cellular proliferation and apoptosis	Downregulated expression of miR-137, miR-193a, miR-133a, miR-133b, miR503 or miR-15a;
		Upregulated expression of miR-21, miR-24 or miR-184
	Metastasis	Downregulated expression of miR-222 or miR-138
		upregulated expression of miR-211 or miR-31
	Chemoresistance	downregulated expression of miR-21
		upregulated expression of miR-23a, miR-214, miR-98
Histone deacetylation	Cell cycle control	Histone deacetylase

loss-of-function is responsible for defect in DNA repair and checkpoints.[13] Methyl groups are transferred to aminoacids of histone proteins that makeup nucleosome by the process of histone methylation. The possible mechanism in histone methylation in carcinogenesis is by loss of cellular memory, i.e., loss of heritable patterns of genetic expression. Consequences of loss of cellular memory are genomic instability, and influence the cancer initiation by self-sufficiency of growth promoting signal. [14] Serine residues that are located on the N-terminal tails of each histone are phosphoreceptor site. Histone Phosphorylation is the addition of phosphate to the histone protein at serine residue region, and is essential in various physiological processes which include chromatin remodelling, intracellular signalling and transcription. Altered histone phosphorylation predisposes a cell with genomic instability and enhanced cellular proliferation. Histone ubiquitination refers to addition of ubiquitin molecule in histone protein, which is responsible for alteration of chromatin structure leading to either activate or inhibit transcription process.[16]

RNA mediated modifications

RNA interference or non-coding RNA is identified to have a significant role in maintenance of gene transcription. MicroRNAs (miRNAs) are non-coding 20-22 nucleotides long RNA which has a critical role in cellular development, proliferation, differentiation and cell death. [17] Most miRNA are located in the genomic loci called fragile sites, and are potential for loss of amplification. miRNA act as mediators of epigenetic regulation by interacting with mRNA by inhibiting mRNA translation or mRNA degradation. miRNAs were reported to act as tumour suppressor oncogenes, epigenetic silencing in cancer. Molecular profiling of miRNA in malignant tissues may reveal as developmental lineage and differentiation status and serve as an instrument in cancer diagnosis and prognosis. miRNA amplification and deletion are the two most common epigenetic alterations under RNA mediated modification. miRNA amplification function as an oncogenes and lead to cancer development. miRNA deletion function as tumour suppressor gene and lead to cancer initiation and development.

Loss of Imprinting

Genomic imprinting refers to the specific locus expression of a very limited set of genes (50-80) from either maternal or paternal genome in the offspring. Genomic imprinting has a pivotal role in developmental, cellular processes. Loss of imprinting due to the epigenetic modification will lead to abnormal biallelic expression and result in syndromes. Pathological biallelic expressions by loss of imprinting mechanism are associated with malignant changes. [19]

Epigenetic dysregulation in oral epithelial carcinomas.

The complexity of epigenetic mechanism and its modification in cancer are extensively studied. Although adequate numbers of research papers have been identified in cancer studies, DNA hypermethylation, histone acetylation, histone deacetylation, and miRNA have been widely investigated in Oral Squamous cell carcinoma tissues. More than 40 hypermethylated genes are reported in oral squamous cell carcinoma tissues literature. These hypermethylated genes that have been identified are related to wide cellular functions such as cell cycle control (p16,15), apoptosis (p14,DAPK, p73, and RASSFIA), Wnt signalling (APC, WIF1, RUNX3), cell to cell adhesion (E-cadherin, EDNRB), DNA repair (MGMT, hMLH1), tumour suppressor (PTEN, ATM, C/EBPa,HIN1, Rb) and epithelial mesenchymal interactions (TCF21, TIMP3, THBS1).[20,21,22,23,24]

The dysregulatedmiRNAs and their cellular function in oral squamous cell carcinoma include proliferation and apoptosis (downregulated expression of miR-137, miR-193a, miR-133a, miR-133b, miR503 or miR-15a; upregulated expression of miR-21, miR-24 or miR-184); metastasis (Downregulated expression of miR-222 or miR-138; upregulated expression of miR-211 or miR-31); chemoresistance (downregulated expression of miR-21; upregulated expression of miR-23a, miR-214, miR-98). [6] Higher expression of histone deacetylase (HDAC) are identified frequently in oral squamous cell carcinoma tissue samples and can be correlated with prognosis of disease. [25,26]

Future Directions:

Epigenetic changes are instrumental in cancer disease identification and to provide therapeutic intervention. Determination of reliable epigenetic change can be employed as biomarker, thereby providing the information on cancer diagnostics, metastasis predictor, disease progression or therapeutic monitoring. However,

future researchers should focus on identification of valid and reliable biomarkers from epigenetic category. Epigenetic alterations are potentially reversible which allow an oncologist to consider these changes as targets for therapeutic intervention. Current research evidence demonstrate that DNA methyltransferases (DNMT) such as 5-azacitidine, decitabine, histone deacetylase are successfully tested and used as a chemotherapeutic agents in treatment of various cancers which include head and neck cancers. The information on Azacitidine and Cisplatin administration in oral squamous cell carcinoma are not available in the literature. Future clinical trial studies from various geographical locations on such drugs targeting epigenetic changes in Oral squamous cell carcinoma are important to address.

Conclusion

It is a well-established concept that epigenetic changes have a promising role in cancer process. Understanding the complexity of epigenetic changes in the cancer biology will help a researcher to focus on diagnostic and therapeutic application in oral squamous cell carcinoma. Identification of valid and reliable biomarker from epigenetic category will switch an instrumental position in screening, diagnosis or therapeutic monitoring. Future epigenetic research may possibly provide new strategic and policy making findings in oral cancer research.

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