Epigenetics in oral cancer- neoteric biomarker

Sameera Shamim Khan[1,*], Mala Kamboj[2], Renuka Verma[3], Malay Kumar[4]

[1,3,4]Senior Lecturer, Career PG Institute of Dental Sciences & Hospital, Lucknow, [2]Professor & Head, Post Graduate Institute of Dental Sciences, University of Health Sciences, Rohtak, Haryana,

***Corresponding Author:**
Email: sameerashamim@yahoo.com

Abstract

A cancer develops when a cell acquires specific growth advantages through the stepwise accumulation of heritable changes in gene function. Cancer genes may be changed by several mechanisms, which potentially alter the protein encoding nucleotide template, change the copy number of genes or leads to increased gene transcription. Epigenetic alterations are increasingly being recognized for their roles in carcinogenesis. Deregulation of gene expression is a hallmark of cancer. Although genetic lesions have been the focus of cancer research for many years, it has become increasingly recognized that aberrant epigenetic modifications also play major roles in the tumorigenic process. These modifications imposed on chromatin, do not change the nucleotide sequence of DNA, and are manifested by specific patterns of gene expression. The field of cancer epigenetics is evolving rapidly on several fronts. Advances in our understanding of chromatin structure, histone modification, and transcriptional activity and DNA methylation have resulted in an increasingly integrated view of epigenetics. Whilst genetic alterations in oral cancer have long been documented, the appreciation of epigenetic changes is more recent. Epigenetic changes alter expression of tumour suppressor genes without changes in DNA sequence. Epigenetic mechanisms such as DNA methylation, histone methylation and deacetylation have been shown to silence key genes involved in cell proliferation, differentiation and genome integrity and clearly have a central role in oral cancer. Epigenetics is another major player in multistep carcinogenesis of oral cancers. In this article we discuss current literature in the field of the epigenetics of oral cancer, placing a great deal of emphasis on DNA methylation, histone modification and post-transcriptional gene down-regulation by microRNAs.

Key words: Cancer, Transcription, Carcinogenesis, Methylation, Epigenetics.

Access this article online	
Quick Response Code:	Website: www.innovativepublication.com
	DOI: 10.5958/2395-6194.2016.00017.5

Definition

Epigenetics literally means "above" or "on top of" genetics. It refers to external modifications to DNA that turn genes "on" or "off." These modifications do not change the DNA sequence, but instead, they affect how cells "read" genes.

Introduction

The transcription of each gene change from high-level expression to complete silencing, depending on the influence of the "epimutations" which interfere with the action of activators and suppressors on specific promoters in the chromatin context. Three major types of epigenetic mechanism are currently known: DNA hypermethylation, histone modification and RNA interference. Disruption of any of these mutually reinforcing epigenetic mechanisms leads to inappropriate gene expression, resulting in cancer development and other "epigenetic diseases"[1-5] (Table 1)

Table 1: Epigenetic Alterations

Epigenetic change	Mechanism	Result
DNA hypomethylation	Activation of cellular oncogenes	Increased proliferation
DNA hypermethylation	De novo hypermethylation of CpG islands within gene promoters	Genomic & chromosomal instability
Loss of imprinting	Biallelic expression of imprinted genes	Expansion of precursor cell population
X chromosome inactivation	Mechanisms unknown	Altered gene dosage
Histone acetylation	Gain of function	Activation of tumor promoting genes
Histone deacetylation	Silencing of tumor suppressor genes	Genomic instability
Histone methylation	Loss of heritable patterns of gene expression	Genomic instability

DNA Methylation

Methylation is the biochemical addition of a methyl group (-CH3) to a molecule and it is referred as methylation of DNA, RNA and proteins. DNA methylation is the most common epigenetic modification.[6] DNA methylation has been studied in oral squamous cell carcinoma (OSCC), in normal tissues, unmethylated cytosine is found in high densities in CpG islands (areas with concentration of cytosine and guanine) that map close to a promoter region in 40% of mammalian genes. This unmethylated state is associated with a high rate of transcriptional activity. This process has been detected in OSCC as a hallmark of cancer. Hypermethylation of p16 occurs in 50–73% of cases and p15 in 60%. Genes showing hypermethylation in OSCC are following

1. CDH1 (cadherin 1 Type 1, a gene on chromosome 16q22.1) produces E-cadherin, which play role in cell adhesion and inhibition.
2. DAPK1 (death-associated protein kinase-1), on chromosome 9q22 associated with apoptosis
3. RARB2 gene (retinoic acid receptor B2) on chromosome 3p24 codes for the nuclear receptor that suppresses transcription activation and cell proliferation.

Hypermethylation of p14ARK, p16 INK4a, P15, MGMT, DAPK, GSTP1 and RARB have been seen in dysplasias and in histologically normal appearing margins of OSCC resections.[7]

Loss of function of tumor-suppressor genes occuring in tumor is ascribed more frequently to epigenetic silencing through methylation than to genetic defects.[6,8] (Table 2)

Table 2: Genes Silenced From Promoter Methylation

Gene	Function	Alterations
ABO	Blood group antigen	Hypermethylation
ATM	Tumor suppressor	Hypermethylation
C/EBPα	Tumor suppressor	Hypermethylation
DAPK	Apoptosis	Hypermethylation
E-cadherin	Signal transduction	Hypermethylation
P 14	Apoptosis	Hypermethylation
P 15	Cell cycle	Hypermethylation

Unequivocal evidence indicates that DNA methylation is closely related to OSCC tumorigenesis.[9] The methylation of DNA refers to the covalent addition of a methyl group to the 5-carbon (C5) position of cytosine bases that are located 5' to a guanosine base in a CpG Dinucleotide. CpG dinucleotides, scattered throughout the genome, are usually found clustered in 0.5–4 kb regions, named CpG islands, the major part of which localized at the promoter of tumor suppressor genes. CpG islands of growth-regulatory genes promoter regions are often found hypermethylated in tumors, this event causing the transcriptional "silencing" of tumor suppressor genes, contribute to cancer progression.[10-12] Hypermethylation is seen with relatively high frequency in OSCC as well as at tumor margins.[7] The development of therapeutics that reverse epigenetic alterations in cancer cells, along with prognostic and diagnostic assays based on gene-methylation patterns, are promising new avenues for future improvements in patient care.

Histone Modification

The basic unit of chromatin is the nucleosome which comprises 146 base pairs of DNA surrounding a histone octomer.[13] Chromatin structure is highly regulated by complex interactions between molecular pathways which influence normal and tumor cell fate, for DNA replication, transcription, and repair, cell growth and differentiation, apoptosis and various cell functions. Histones and chromatin modifiers induce changes of chromatin architecture and play role in the chromatin architecture entering into the constitution of nucleosomes.[14,15] Post-translational modifications of histones are observed in oral cancer. These epigenetic alterations occur primarily at the N-terminal tails within each of the four histone complexes (H3, H4, H2A and H2B). Other modifications include methylation, acetylation, phosphorylation, ubiquitination and sumoylation of specific residues within these histone tails. These processes are generally reversible and modify the tertiary DNA structure.[16] Histone methylation, mediated by histone methyltransferases (HMTs), have positive or negative effects on gene expression. Increase in histone acetylation correlates with gene activation, and results from dynamic interplay between histone acetyltransferases (HATs) and histone deacetylases (HDACs). The ultimate mediators of histone methylation associated gene silencing appear to be proteins that bind specific modified histone and recruit effector protein complexes. Among these, the ones which seems to play a role in carcinogenesis belongs to ING protein family. The ING proteins are involved in cell cycle and apoptosis. Recently, the potential roles of ING proteins as prognostic biomarkers, detector of aggressive behavior of tumors as well as factor of chemoradiotherapy response, have been hypothesized.[17] Methylation of specific lysine residues within histone tails is now associated with either activation or transcriptional repression of gene transcription. However, this is primarily dependent upon the position of the specific lysine residue being methylated, as well as the number of sites methylated. However the process of DNA methylation and histone methylation are tightly coregulated and collectively play an important role in carcinogenesis.

RNA Interface

It is a system involved in controlling gene activation in living cells. There are two types of small RNA molecules – microRNA (miRNA) and small interfering RNA (siRNA).

Micro-RNAs (miRNAs) was first identified in Caenorhabditis in 1993, they are small non-coding RNAs, which play role in modifying genes expression, composed of 20–22 nucleotides, typically excised from 60–110 nucleotide fold back RNA precursor structures.[17]miRNAs are the most recent entrants into the category of epigenetic gene expression regulators it represent small RNA molecules with important regulatory functions,

Where in each miRNA has the potential to target multiple mRNAs or gene targets. miRNAs Regulate gene expression on a post-transcriptional level, inhibiting protein formation by Degradation or repression of translation of the mRNA transcript.[18] They act as mediators of epigenetic gene regulation, by interacting with mRNA, either by inhibiting mRNA translation or causing mRNA degradation. Recent studies have been shown that miRNAs act as putative tumor suppressors and may also undergo epigenetic silencing in cancer.[19-21] During recent years, the trend of research in mi-RNA and OSCC field has evolved from solely searching altered specific miRNAs to exploring molecular networks and connections between miRNAs and signaling pathways involved in the progression of OSCC.

Small interfering RNAs (siRNA) also referred to as **short interfering RNAs** or **silencing RNAs** represent a class of double-stranded RNA molecules, 20-25 nucleotides in length, that play a notable role in the RNA interference (RNAi) pathway, where it interferes with the expression of a specific gene, but also in RNAi-related pathways as well as in antiviral mechanism or in shaping the chromatin structure of a genome. siRNAs were first discovered by David Baulcombe's group in Norwich, England, as part of post-transcriptional gene silencing (PTGS) in plants. This discovery led to a surge in interest in harnessing siRNA for biomedical research and drug development. It is expected that in some situations turning off or knocking down the activity of a gene with an siRNA could produce a therapeutic benefit.[17]

Epigenetic Therapy

The potential reversibility of epigenetic states offers opportunities for novel cancer drugs that can reactivate epigenetically silenced tumor-suppressor genes. Blocking either DNA methyltransferase or histone deacetylase activity could potentially inhibit or reverse the process of epigenetic silencing. DNA methyltransferases and histone deacetylases are the two major drug targets for epigenetic inhibition to date, although others are expected to be added in the near future. The network of multiple reinforcing interactions involved in epigenetic silencing suggests that combination therapy would be a particularly appropriate strategy to achieve clinical efficacy. Indeed, combinations of DNA methyltransferase and histone deacetylase inhibitors appear to synergize effectively in the reactivation of epigenetically silenced genes.

Future Trends

Epigenetic mechanisms have emerged as important contributors in the pathogenesis of various human cancers. More importantly, since these alterations occur frequently in the process, and are potentially reversible, this makes them ideal for exploitation as disease biomarkers as well as therapeutic targets in human cancer. It is just a matter of time before most of these approaches are put into everyday clinical practice, which will undoubtedly help reduce oral cancer morbidity and mortality in the not-so-distant future.

References

1. Santos-Rosa, H.; Caldas, C. Chromatin modifier enzymes, the histone code and cancer. Eur. J. Cancer 2005,41,2381–2402.
2. Rountree, M.R.; Bachman, K.E.; Herman, J.G.; Baylin, S.B. DNA methylation, chromatin inheritance, and cancer. Oncogene 2001,20,3156–3165.
3. Egger, G.; Liang, G.; Aparicio, A.; Jones, P.A. Epigenetics in human disease and prospects for epigenetic therapy. Nature 2004,429,457–463.
4. Feinberg, A.P.; Ohlsson, R.; Henikoff, S. The epigenetic progenitor origin of human cancer. Nat. Rev. 2006,7,21–33.
5. Feinberg, A.P.; Tycko, B. The history of cancer epigenetics. Nat. Rev. Cancer 2004,4,143–153.
6. Jones, P.A.; Baylin, S.B. The fundamental role of epigenetic events in cancer. Nat. Rev. Genet.2002,3,415–428.
7. MW Lingen, A Pinto, RA Mendes, R Franchini, R Czerninski, WM Tilakaratne8, M Partridge, DE Peterson, S-B Woo. Oral Diseases (2011) 17 (Suppl. 1),7–22.
8. Herman, J.G. Hypermethylation of tumor suppressor genes in cancer. Semin. Cancer Biol. 1999,9,359–367.
9. Piyathilake CJ, Bell WC, Jones J, et al. Pattern of nonspecific (or global) DNA methylation in oral carcinogenesis. Head Neck. 2005;27(12):1061–1067.
10. Bird, A. DNA methylation patterns and epigenetic memory. Genes Dev. 2002,16, 6–21.
11. Eden, A.; Gaudet, F.; Waghmare, A.; Jaenisch, R. Chromosomal instability and tumors promoted by DNA hypomethylation. Science. 2003,300.
12. Bonazzi, V.F.; Irwin, D.; Hayward, N.K. Identification of candidate tumor suppressor genes inactivated by promoter methylation in melanoma. Genes Chromosom. Cancer 2009,48,10–21.
13. FEINBERG AP. Cancer epigenetics takes center stage. Proc. Natl. Acad. Sci. U.S.A 2001:98:392-394.14.
14. Jenuwein, T.; Allis, C.D. Translating the histone code. Science 2001,293,1074- 1080.
15. Strahl, B.D.; Allis, C.D. The language of covalent histone modifications. Nature 2000,403,41–45.
16. Volkel P, Angrand PO. The control of histone lysine methylation in epigenetic regulation. Biochimie. 2007;89(1):1–20.

17. A. Santoro1, G. Pannone, S. Papagerakis, R. Serpico, A. Guida, L. Lo Muzio and P. Bufo. Epigenetic Profiling of Oral Cancer.
18. Wong TS, Liu XB, Wong BY, Ng RW, Yuen AP, Wei WI. Mature miR-184 as potential oncogenic microRNA of squamous cell carcinoma of tongue. Clin Cancer Res. 2008;14(9):2588–2592. miRNA profiling study on tongue squamous cell carcinoma and normal tissues examining the expression level of 156 miRNAs.
19. Sood, P.; Krek, A.; Zavolan, M.; Macino, G.; Rajewsky, N. Cell-type-specific signatures of microRNAs on target mRNA expression. Proc. Natl. Acad. Sci. USA 2006,103,2746–2751.
20. Lujambio, A.; Ropero, S.; Ballestar, E.; Fraga, M.F.; Cerrato, C.; Setién, F.; Casado, S.; Suarez-Gauthier, A.; Sanchez-Cespedes, M.; Git. A.; et al. Genetic unmasking of an epigenetically silenced microRNA in human cancer cells. Cancer Res. 2007,67,1424–1429.
21. Saito, Y.; Liang, G.; Egger, G.; Friedman, J.M.; Chuang, J.C.; Coetzee, G.A.; Jones, P.A. Specific activation of microRNA-127 with down regulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells. Cancer Cell 2006,9,435–443.