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Mini Review

Distinct DNA Methylation Patterns in Oral Squamous Cell Carcinoma

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Abstract

Oral Squamous Cell Carcinoma (OSCC) is characterized by a broad spectrum of genomic imbalances, including gross chromosomal alterations, such as polysomy/aneuploidy and specific gene aberrations. Concerning the development of OCSSC, broad clinic-molecular studies have recognized chronic tobacco, alcohol and also betel quid consumption combined or not with persistent viral infections -especially High-Risk Human Papilloma Virus (HR HPV)-as main etio-pathogenetic factors. Oncogene and suppressor gene deregulation due to amplification, point mutations and loss of heterozygosity combined or not with epigenetic changes, such as promoter methylation are responsible for the progressive transformation of normal squamous epithelia to neoplastic and finally malignant. Concerning aberrant methylation, a variety of genes and DNA sites has been identified implicated in OSCC rise and progression. Distinct methylation patterns seem to be associated with biological behaviour of the malignancy and altered response to specific chemotherapy agents in the corresponding patients. These epigenetic changes should be potentially useful biomarkers for molecular discrimination of patients suffering by OSCC. This review summarises the different epigenetic aspects -regarding predominantly to methylation alterations- detected in OSCC and their impact in the corresponding groups of patients.

Keywords: Oral; Carcinoma; Gene; Epigenetics; Methylation

Introduction

Since last three decades, a remarkable evolution in molecular biology and oncology has been achieved expanding the limits of our knowledge in the field of carcinogenesis. Mechanisms and multistep processes have been identified based on extensive genetic and epigenetic analyses. Their combination has shown that cancer genome consists of a variety of genetic and epigenetic alterations that modify normal DNA/m RNA sequences triggering a cataract of reactions inside and outside the nucleus micro-environment [1]. Gross chromosomal and specific gene alterations are genetic are involved in its rise, progression and metastatic expansion [2]. Concerning solid tumors, a variety of gene functional and numerical imbalances in crucial molecular pathways such as cell cycle regulation, signaling transduction, apoptosis or angiogenesis have been identified and explained [3]. Cell malignant transformation is mediated by an aberrant gene expression, including predominantly oncogenes up regulation combined with suppressor genes down regulation that lead to cell cycle deregulation [4]. Point mutations, polymorphisms, abnormal gene copy number (amplification, deletion), or structural chromosomal rearrangements (translocations) and epigenetic modifications including aberrant methylation detectable by different molecular techniques provide critical information to oncologists for handling those patients in a rational therapeutic way regarding their isolated molecular landscape and the corresponding specific genetic signatures [5]. Among Head and Neck Squamous Cell Carcinomas (HNSCCs), Oral Squamous Cell Carcinoma (OSCC) is characterized by a broad spectrum of genomic imbalances, including gross chromosomal alterations, such as polysomy/aneuploidy and specific gene aberrations. Concerning the development of OCSSC, main factors are chronic tobacco, alcohol and also betel quid consumption combined or not with persistent viral infections, especially High Risk Human Papilloma Virus (HR-HPV) related [7]. This review is focused on the different methylation aspects detected in OSCC and their impact in the corresponding groups of patients.

Epigenetic Mechanisms in Carcinogenesis

Epigenetic modifications are referred to specific biochemical changes in the genome of a cell leading to altered gene activity-mainly silence- and expression. In contrast to genetic changes, they do not affect the entire DNA nucleotide sequences [8]. Similarly to genome, epigenome represents a synthesis of all biochemical compounds and mechanisms that modify gene expression profiles. Epigenome comprises specific chemical reactions including methylation (hyper-, hypo), histone modifications (acetylation), micro-RNAs (miRs) alterations and also chromatin re-organization. Concerning methylation procedure, it is based on the insertion of one or multiple new methyl groups (CH_3) in the 5' position of cytosine residues at Cytosine-Phosphate-Guanosine Dinucleotide Areas (CpG islands) provided by the activity of specific DNA enzymes, the methyltransferases [9]. Induced and accumulated methylation (hypermethylation) in areas of high significance - such as gene promoter regions especially in tumor suppressor genes- are responsible for their functional inactivation, whereas hypomethylation leads to oncogene over activation. Hypo- and hyper-methylation seem to

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be early genetic events in the carcinogenetic process, but the first pattern mainly has been detected to be combined with chromosomal instability [10]. In contrast, hypermethylation and also histone hypoacetylation are co-involved in tumor suppressor genes silencing [11]. Besides the previous described epigenetic changes that are involved in carcinogenetic process, microRNas (miRs) demonstrate an increasing interest for understanding their role in cancer and also in handling patients via targeted therapeutic agents [12]. miRs are short, non-coding RNA molecules consisting of 20-25 nucleotides located at intra- or inter-gene regions [13]. RNA polymerase II is responsible for their transcription. Initially, pri-miRNAs are reformed to pre- miRs followed by a maturation process. In the nucleus, the RNase III enzyme Drosha complex provides release of the pre-miRs to the cytoplasm where the final single-stranded mature miR is produced [14]. Functional miRs mediate a positive regulation of posttranscriptional gene silencing. miRNA deregulation in cancerous cells due to genetic (mutations, translocations), epigenetic (DNA hypermethylation of tumour suppressor genes, extensive genomic DNA hypomethylation, aberrant histone modification patterns) and transcriptional alterations leads to a loss of miR-mediated repression of target mRNA [15-17]. Interestingly, a biphasic role of miRs in cancers of different histogenetic origin has been detected. In some of them, their up regulation seems to correlate with increased ongogenic activity, whereas in others the same miRNA type acts as a suppressor agent (miRNA 29 in hepatocellular carcinoma and lung cancer, miRNA 26a in lung and breast lung cancer, respectively) [18,19].

DNA Methylation Changes and Mechanisms in OSCC

Detecting specific epigenetic changes in pre- and malignant oral epithelia is the first step in understanding their impact in the onset and progression of OSCC. Distinct epigenetic patterns include cytosine methylation in CpG islands and also histone posttranslational modifications as a result of phosphorylation, deacetylation and ubiquitinylation aspects [20]. Tumor suppressor genes' silencing -due to their transcriptional repression- is also mediated by aberrant methylation of the corresponding promoter regions and represents a critical mechanism in OSCC aggressive biological behavior [21]. Extensive molecular studies based on specific and accurate techniques-such as pyrosequencing analysis assays- have identified methylated CpG sites in a series of screened genes in OSCC tissues including the FLT4, KDR, and TFPI2, respectively [22]. Additionally, another study implementing also pyrosequencing analysis detected a unique methylation site (cg01009664) in the Thyrotropin-Releasing Hormone (TRH) gene [23]. They also suggest that this methylation pattern could be useful as an epigenetic biomarker of OSCC. Using integrated methylation and gene expression microarray analysis, another study group reported a biphasic pattern of methylated genes (highly hypermethylated or hypomethylated) in a series of OSCCs correlated also to survival status of the corresponding patients [24]. This specific methylation set comprised Fibroblast Activation Protein$^{\alpha}$ (FAP), Interferon A Inducible Protein27 (IFI27), Laminin Subunit Γ^{2}(LAMC2), Matrix Metallopeptidase1 (MMP1), Serine Peptidase Inhibitor Kazal Type 5 (SPINK5) And Zinc Finger Protein 662 (ZNF662) genes, respectively. Concerning the micro-molecular differences between oral premalignant lesions and OSCC, another experimental study based on bisulfite next-generation sequencing methylation analysis reported a set of genes that should be used for discriminating the two entities [25]. Interestingly, hypermethylation was detected in ZAP70, ITGA4, KIF1A, PARP15, EPHX3, NTM,LRRTM1, FLI1, MIR193, LINC00599, PAX1, and MIR137HG, whereas MIR296, TERT, and GP1BB genes demonstrated hypomethylation. Similarly, another set of methylated genes regarding OSCC has been also analyzed. SFRP2 and RASSF1A genes were found to be hypomethylated, whereas RARβ and DAPK1 showed higher methylation rates [26]. Both of them correlated to advanced lymph node metastasis (N stage), whereas. Furthermore, DAPK1 hypermethylation had a positive impact on death risk in patients (extended lifespan). Novel techniques including targeted multiplex bisulfite amplicon sequencing have also detected gene methylated sets composing new CpG methylated sites landscape in OSCC and also in pharyngeal squamous cell carcinoma [27]. Concerning tumour suppressor genes deregulation in OSCC, p16 has been also analyzed at the level of altered methylation. A study group concluded that HPV positive OSCC tissues demonstrate a high frequency of p16 promoter methylation and down regulation compared to HPV negative ones associated also to an aggressive malignant phenotype [28]. Besides p16, Sal-Like Protein 2 (SALL2) promoter methylation seems to be another potential epigenetic biomarker for OSCCs. Inactivation of the gene due to its loss of m RNA expression has been identified in an analyzed series of OSCC [29]. Concerning the methylation level of specific histones in OSCC, a study group reported a progressive elevation of H3K4 histone in leukoplakias and OSCC that leads to an alteration of chromatin structure [30]. In conclusion, a variety of methylation patterns seem to be associated with biological behaviour of the malignancy and altered response to specific chemotherapy agents in the corresponding patients. Promoter hyper-hypomethylation in critical tumour suppressor and onco-genes respectively, deregulate significantly the normal oral epithelia. Aberrant methylation is mainly an early epigenetic change leading progressively to pre- and malignant transformation of the normal mucosa. Understanding the nature and mechanisms of these epigenetic changes is the first step for evaluating their impact as potential useful biomarkers for molecular discrimination of patients suffering by OSCC.

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References

1. Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. Cell 2011; 144: 646-74.
2. Polyak K, Haviv I, Campbell IG. Co-evolution of tumor cells and their microenvironment. Trends Genet. 2009; 25: 30-8.
3. Stratton MR, Campbell PJ, Futreal AP. The cancer genome. Nature 2009; 458: 719-24.
4. Schlessinger J. Cell Signaling by Receptor Tyrosine Kinases. Cell 2000; 103: 211-25.
5. Albertson DG, Collins C, McCormick F, Gray JW. Chromosome aberrations in solid tumours. Nat Genet 2003; 34: 369-76.
6. Kallionemi A. Comparative genomic hybridization for molecular cytogenetic analysis of solid tumours. Science 1992; 258: 818-21.
7. Grade M, Difilippantonio MJ, Camps J. Patterns of Chromosomal Aberrations in Solid Tumors. Recent Results Cancer Res. 2015; 200: 115-42.
8. Ali J, Sabiha B, Jan HU, Haider SA, Khan AA, Ali SS. Genetic etiology of oral cancer. Oral Oncol. 2017; 70: 23-28.
9. Patel V, Leethanakul C, Gutkind JS. New approaches to the understanding of

the molecular basis of oral cancer. Crit Rev Oral Biol Med. 2001; 12: 55-63.

10. Okafuji M, Ita M, Oga A, Hayatsu Y, Matsuo A, and Shinzato Y. The relationship of genetic aberrations detected by comparative genomic hybridization to DNA ploidy and tumor size in human oral squamous cell carcinomas. J Oral Pathol Med 2000; 29: 226-3.

11. Ehrlich M. DNA hypomethylation in cancer cells. Epigenomics 2009; 1: 239-59.

12. Jansson MD, Lund AH. MicroRNA and cancer. Mol Oncol. 2012; 6: 590-610.

13. Bartel DP. Micro RNAS: target recognition and regulatory functions. Cell 2009; 136: 215-33.

14. Saj A, Lai EC. Control of microRNA biogenesis and transcription by cell signaling pathways. Curr. Opin. Genet. Develop. 2011; 21: 504-10.

15. Lee Y, Kim M, Han J. MicroRNA genes are transcribed by RNA polymerase II. EMBO J. 2004; 23: 4051-60.

16. Mishra PJ, Mishra PJ, Banerjee D, Bertino JR. MiRSNPs or MiR-polymorphisms, new players in microRNA mediated regulation of the cell: introducing microRNA pharmacogenomics. Cell Cycle. 2008; 7: 853-8.

17. Gebeshuber CA, Zatloukal K, Martinez J. miR-29a suppresses tristetraprolin, which is a regulator of epithelial polarity and metastasis. EMBO Rep. 2009; 10: 400-5.

18. Croce CM. Causes and consequences of microRNA dysregulation in cancer. Nat Rev. Genet. 2009; 10: 704-14.

19. Liu, B, Wu X, Liu B. MiR-26a enhances metastasis potential of lung cancer cells via AKT pathway by targeting PTEN. Biochim. Biophys Acta. 2012; 1822: 1692-704.

20. García MP, García-García A. Epigenome and DNA methylation in oral squamous cell carcinoma. Methods Mol Biol. 2012; 863: 207-19.

21. González-Ramírez I, García-Cuellar C, Sánchez-Pérez Y, Granados-García M. DNA methylation in oral squamous cell carcinoma: molecular mechanisms and clinical implications. Oral Dis. 2011; 17: 771-8.

22. Li YF, Hsiao YH, Lai YH, et al. DNA methylation profiles and biomarkers of oral squamous cell carcinoma. Epigenetics. 2015; 10: 229-36.

23. Puttipanyalears C, Arayataweegool A, Chalertpet K, et al. TRH site-specific methylation in oral and oropharyngeal squamous cell carcinoma. BMC Cancer. 2018; 18: 786-92.

24. Zhao C, Zou H, Zhang J, Wang J, Liu H. An integrated methylation and gene expression microarray analysis reveals significant prognostic biomarkers in oral squamous cell carcinoma. Oncol Rep. 2018; 40: 2637-47.

25. Morandi L, Gissi D, Tarsitano A, Sofia A, Andrea G, Claudio M. et al. CpG location and methylation level are crucial factors for the early detection of oral squamous cell carcinoma in brushing samples using bisulfite sequencing of a 13-gene panel. Clin Epigenetics. 2017; 15: 85-90.

26. Strzelczyk JK, Krakowczyk Ł, Owczarek AJ. Methylation status of SFRP1, SFRP2, RASSF1A, RARβ and DAPK1 genes in patients with oral squamous cell carcinoma. Arch Oral Biol. 2019; 98: 265-72.

27. Langevin SM, Kuhnell D, Niu L, et al. Comprehensive mapping of the methylation landscape of 16 CpG-dense regions in oral and pharyngeal squamous cell carcinoma. Epigenomics. 2019; 11: 987-1002.

28. Allameh A, Moazeni-Roodi A, Harirchi I, et al. Promoter DNA Methylation and RNA Expression Level of p16 Gene in OralSquamous Cell Carcinoma:Correlation with Clinicopathological Characteristics. Pathol Oncol Res. 2019; 25: 1535-43.

29. Imai A, Mochizuki D, Misawa Y, et al. SALL2 Is a Novel Prognostic Methylation Marker in Patients with Oral SquamousCarcinomas: Associations with SALL1 and SALL3 Methylation Status. DNA Cell Biol. 2019; 38: 678-87.

30. Mancuso M, Matassa DS, Conte M, et al. H3K4 histone methylation in oral squamous cell carcinoma. Acta Biochim Pol. 2009; 56: 405-10.