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Title: Epigenetics and its implications for oral health

Amarjot Singh[1], Harpal Jakhu[2,4], Gurveen Gill[1,3]

1. **Faculty of Dentistry, McGill University, Montreal, Quebec, CANADA**
2. **Department of Endodontics, Government Dental College, Amritsar, Punjab, INDIA**
3. **Lady Davis Institute, Jewish General Hospital, Montreal, Quebec, CANADA**
4. **Sandalwood Smiles, Private Dental Practice, Brampton, Ontario, CANADA**

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Correspondence should be addressed to:
Amarjot Singh, BDS, MSc.
Faculty of Dentistry, McGill University,
Montreal, QC, CANADA
+1-514-802-5672
amarjot.singh@mail.mcgill.ca

Abstract:

Background: Over the last decade, we have been able to understand the role of various genetic processes involved in the oral diseases. However, In past few years, much of stresses has been paid to understand the regulation of these genetic processes controlled by the epigenetic mechanisms. Epigenetic processes regulate the gene activity without altering underlying DNA sequences, through mechanisms including DNA methylation, histone modifications, and non-coding RNAs. These epigenetic processes are significantly associated with various oral health problems including periodontitis, oral cancer.

Highlights: Different strategies like Genome-wide DNA methylation studies, microRNA based profiling studies, next-generation RNA sequencing analyses have been proven to be helpful in establishing the relationship among these epigenetic processes and gene expression. A few studies also suggest an emerging association of various epigenetic biomarkers in oral cancer and periodontal diseases.

Conclusion: Epigenetic mechanisms are highly sensitive and reversible, thereby offers significant potential for their use to develop novel diagnostic tools, pharmacotherapies and personalized based treatment care. However, there is lack of integrated approaches combining epigenomics with proteomics and genomics comparing the usefulness of epigenetic biomarkers in oral health research.

Keywords: Epigenetics, Periodontitis, Oral Cancer, Biomarkers, Personalized Medicine

1) Introduction:

The advancements in the cellular and molecular biology have played a tremendous role in our understanding of the complex clinical disorders. Epigenetics is one of the fields, which has been studied extensively in various areas of health research like neurosciences, cancer medicine etc. The term epigenetics was coined in 1942 which refers to changes in the gene expression that results from external or environmental factors, and such changes are not encoded in DNA sequence[1].

The DNA encodes the secrets of life in two specific layers of information. The first layer of information which forms the basis of our genetic code is called the coding region of DNA. This coding region comprises only 2% portion of total DNA and remaining 98% region is called non-coding region. The genetic code is translated to different mRNAs and is responsible for creating all the proteins that we need to function. The second layer which is above this genetic code known as the epigenetic layer is responsible for deciding when, where and which gene to express. This epigenetic layer comprises the different epigenetic mechanisms including DNA Methylation, Histone Modifications or non-coding RNAs. Epigenetic mechanisms are crucial for cellular differentiation, and this process is highly conserved and controlled at the cellular level in each particular organism. When such highly conserved process is affected by the external environment directly or indirectly, it leads to change in the expression of genes and thus function. For example, differentiation of human CD4+ T cells is influenced by the locally acting inflammatory substances[2]. These inflammatory substances or interleukins further activate transcription factors that bind to specific methylated or demethylated sites in the promoter regions of specific genes in a developing T cell DNA and decide the fate of lineage of T cell

development[3]. The increase in DNA methylation close to the promoter region of the specific gene results in decreased expression and vice versa[3]. Similarly, in pathological conditions like periodontitis, the local immune response is decided by these locally acting transcription factors and methylation or demethylation of specific genes[4].

The recent development of next-generation sequencing technologies and genome-wide methylation studies have been proven to be helpful to determine the mechanisms in different pathological conditions including neurological disorders, cancer etc.. In addition to that, epigenome-wide association studies (EWAS) across the human population has played a key role in uncovering the novel molecular mechanism, development of biomarkers in various diseases like cancer. In this review, we will cover the detail of the epigenetic mechanisms and their applications as biomarkers and personalized medicine in oral health or dental medicine.

1.1 DNA Methylation:

DNA Methylation is one of the most extensively studied epigenetic mechanism which stands for the addition of the Methyl (-CH3) group to the Carbon 5 of pyrimidine ring of cytosine (C) nucleotide of DNA under the action of enzymes DNA methyltransferases (DNMTs) (Figure 1). This covalent modification of DNA is most susceptible to Cytosine bases lying in conjunction with Guanine bases, termed as C-phosphate-G or CpG groups. These CpG sites are 70-80 % methylated in mammals. In mammals, the enzymes to catalyzes these reactions are DNA Methyl Transferase (DNMT 1, 3a, 3b). DNMT1 is responsible for maintaining the methylation states whereas DNMT3a, 3b are responsible for the de novo methylation induced during different conditions. Methylated CpG sites attract various Methylated-CpG binding proteins (MBDs) such as Methylated-CphosphateG2 (MeCp2). Methylated CpGs attract other

inhibitory transcription factors and results in downregulation of gene expression. This event is more efficient in regulating the gene expression when inhibitory transcription factors bind in proximity to the promoter region or Transcription Start Site (TSS) region[5].

Functionally, this whole event is complex because of the uneven scattering of CpG sites in a nucleotide sequence. Statistically, these CpG sites when differentially methylated in the intervention group as compared to the control group are called as differentially methylated regions or DMRs. Various technologies have been developed to study the DMRs such as bisulfite sequencing, pyrosequencing, Methylated DNA Immunoprecipitation (MeDIP), Next Generation Sequencing (NGS) etc[6,7]. Functionally, to investigate the effect of DNA methylation on gene expression for a specific gene, DMRs in proximity to promoter region are studied for hypermethylation or hypomethylation. For quantification of DNA methylation and gene expression qMeDIP and RT-PCR, technologies are used respectively in conjunction with each other[5].

1.2 Histone Modifications:

Histone modifications through histone acetylation or histone methylation exert a significant impact on chromatin compaction which further results in changes in the gene expression (Figure 1). The N-terminal tails of histones are highly accessible and subject to post-translational modifications, especially at H3 and H4 histone sites. Histone acetylation is thought to be more responsive to environmental stimuli than DNA methylation or histone methylation and therefore, results in more transient cellular modifications resulting in abrupt changes in gene expression[8,9]. Histone acetylation is driven by enzymes known as histone acetyltransferases (HATs) by catalyzing the addition of acetyl group to histone groups. The

addition of acetyl group to histone groups further promote gene transcription. Contrary to it, the removal of an acetyl group from histone groups results in compaction of chromatin and ultimately inhibition of gene transcription. The removal of acetyl groups from histone groups is catalyzed by the enzymes known as histone deacetylases (HDACs). In humans, eleven different types of HDACs have been identified, which are further classified into different groups based on their structure. Class I, II, IV represents zinc-dependent classes and Class III represents the nicotinamide adenine dinucleotide-dependent class. Class I comprises of HDAC1,2,3 and eight whereas Class II is further divided into Class IIa (HDAC 4,5,7 and 9) and Class IIb (HDAC 6 and 10)[10,11].

1.3 Non-Coding RNAs:

Third and last entrants that regulate the epigenetic mechanisms are non-coding RNAs. Non-coding RNAs are functional molecules like mRNA but rather not translated into proteins (Figure 1). Non-coding RNAs are further divided into long non-coding RNAs (ln RNAs) and micro RNAs (miRNAs)[12]. miRNAs contribute to epigenetic machinery by degradation of the specific mRNAs and thus resulting in down-regulation of transcription of the gene[13]. One miRNA can bind to several different mRNAs or genes and thus can modulate the expression of different other gene transcripts. More than 2000 miRNA transcripts have been identified in humans so far[14]. The unique and differential expression of miRNAs signatures is thought to be indicative of particular disease states and thus are implicated to use as diagnostic and prognostic biomarkers[12]. miRNA profiling can be done by first extracting the RNA from specific tissue and then using qPCR, microarray or RNA sequencing. With the development of Next-generation sequencing (NGS) technologies, miRNA profiling studies have been proven to be immensely

useful for their use as diagnostic and prognostic biomarkers in different dental diseases and oral cancer[12,15,16].

2 Epigenetics in relation to periodontal diseases:

2.1 DNA Methylation and periodontitis

In periodontitis, where the inflammation is mainly caused in response to the inflammatory cell products released by the bacteria, epigenetic modifications play a vital role in the regulation of host immune response, especially at the biofilm-gingival interface. Due to modulation of local immune response by the epigenetic processes at inflammatory sites, the epigenome differs from non-inflammatory sites in same individual[17,18]. The DNA methylation signature differs in the individual with periodontitis when compared with healthy individuals[18]. Since the epigenetic modifications are not permanent, they can be reversed by altering the local environment. In other words, clearing the pathogenic bacteria by periodontal procedures such as curettage may change the epigenome of inflammatory regions. These methylation signatures are of high importance in epigenome-wide association studies since they can be used to predict the outcome of disease using as prognostic and diagnostic markers.

Oliviera et al. have investigated the effect of smoking on epigenome in patients with chronic periodontitis. A significant hypomethylation of IL8 gene was observed in oral epithelial cells of non-smokers as compared to patients with smoking history. Comparatively, an increased mRNA expression of IL8 was observed in the gingival epithelial cells of smokers than non-smokers [19]. In a similar study, patients with aggressive periodontitis showed the hypomethylation of CpGs in promoter region when compared with the healthy individuals but mRNA levels were not measured for IL-8 gene[20].

Apart from the cytokines, a number of other genes correspond to the altered DNA methylation levels, suggesting a complex signaling pathway in the pathogenesis of chronic periodontitis. Toll-like receptors TLR2 and TLR4 genes showed a unique pattern of DNA methylation signature in periodontitis. TLR-2 gene was both hypomethylated and hypermethylated at different CpG sites in promoter region whereas TLR-4 was significantly hypomethylated in individuals with periodontitis[21]. In another study, hypermethylation of TLR-2 promoter region was reported with subsequent downregulation of TLR-2 in the intervention group compared to control group[22].

2.2 Histone Modifications and Periodontitis

Since epigenetic modifications may occur with or without conjunction with other epigenetic processes, various studies have focussed on looking collectively at the changes in DNA Methylation as well as histone modification[23,24]. In relation to periodontitis, a few studies have focussed on the histone modifications and Histone Deacetylases (HDACs). Deacetylation of histones results in compaction of chromatin, thus downregulation of gene expression. HDAC inhibitor (HDACi), when used in a mouse model of periodontitis, resulted in suppression of bone loss and hence were implicated in the management of periodontitis[25].

In another study, both methylation and histone modifications were analyzed for IL-10 gene expression in patients with periodontitis[26]. No significant differences were observed in the methylation status of blood and gingiva samples as well as between 1L-10 genotypes (GG and GA). However, a difference in the IL-10 gene expression was seen in genotypes of -1087 site-specific GG and GA polymorphism[26].

2.3 Micro RNA and Periodontitis

A few number of studies have done micro RNA (miRNA) profiling in relation to the periodontal diseases[27–29]. Most of these studies used micro RNA microarray to examine the expression of miRNAs in healthy and gingival tissues. The expression of few selected miRNAs was confirmed then later using real-time or quantitative polymerase chain reaction (RT-PCR). In a study by Xie et al., a significant upregulation of 6 different miRNAs in patients with inflamed gingiva. A few miRNAs showed a five-fold increase in the expression among intervention group. The findings suggested a role of Toll-like Receptors in the pathogenesis of periodontitis using miRNA pathways[28].

In a pilot study by R. Perri et al., miRNA expression was evaluated in obese patients in the presence or absence of periodontitis or both. The study was performed to determine the underlying pathogenesis and relation between periodontitis and obesity. Two miRNAs were identified (miR-18a, miR-30e) to be upregulated in cohort with obese individuals with healthy periodontium. Similarly, two miRNAs (miR30-e, miR106-b) were identified to be upregulated in cohort with obese individuals with periodontitis. In individuals with both obesity and periodontitis, nine miRNAs species were upregulated. A total of 69 mRNA targets were found for these miRNAs which mainly comprises of cytokines and chemokines. These differentially regulated circulating miRNAs are of high significance using as diagnostic and prognostic biomarkers[30].

Micro RNA profiling studies are more meaningful when genome-wide mRNA analysis is performed in conjunction with each other. Since miRNAs target various mRNA, using both technologies, it can be confirmed that which miRNA and mRNA are upregulated. Later on, gene

set enrichment analysis can be performed to find the effective mRNA targets of specific miRNA. Stoecklin-Wasmer et al., conducted a similar kind of combination of these technologies in diseased and healthy gingival tissues. miRNA profiling and mRNA profiling was done using whole genome microarray analysis. The expression level of each mRNA or miRNA was confirmed later on, using RT-PCR[27].

Since the most common application of miRNA profiling studies is regarding their use as biomarkers but certain issues should be considered with respect to dental diseases. Firstly, most of these studies are pilot studies, a more detailed and systematic approach is required to validate further the role of miRNAs as biomarkers. Secondly, the next generation or high throughput screening techniques used for miRNA profiling are lacking validated endogenous control for normalization for salivary samples[31]. Moreover, there is a lack of the studies focussed on exploring the effect of epigenetic changes in the etiology of the periodontal diseases, especially refractory periodontitis.

3 Epigenetics and Oral Cancer

3.1 DNA Methylation in Oral Precancerous and Cancerous Lesions

Various studies have suggested both hypermethylation and hypomethylation as an early event in oral cancer. Hypomethylation is commonly seen in repeat sequences of genome whereas hypermethylation is seen in few candidate genes which are considered as major factors in the development of oral cancer[32]. Among carcinogens, tobacco and its metabolites attribute more than 80% risk in the development of oral cancer and contribute to differential methylation of genes by altering the expression of DNMTs (DNA methyltransferases)[33,34]. Lin et al., studied the tobacco-specific carcinogen nicotine-derived nitrosamine ketone (NNK) which

induced hypermethylation in a number of genes including DAPK and p16. Also, p16 promoter hypermethylation was identified in “Maras powder” and tobacco users[35].

In Oral Squamous Cell Carcinoma (OSCC), genome-wide methylation studies have focussed on identifying aberrant DNA methylation and validating few candidate gene significantly involved (Table1). These studies demonstrated that hypermethylation and hypomethylation at promotor region of few candidate genes(mentioned in Table 1) may have been involved in the pathogenesis of OSCC, thus may serve as a promising tool in diagnostic and prognostic cancer medicine. However, each of these studies was performed independently on various ethnic groups with different geographical locations. There is the possibility of difference in epigenetic signatures among these groups. Hence, Epigenome-wide association studies (EWAS) are required to single out the unique DNA methylation patterns contributing towards OSCC.

Hypomethylation and overexpression of HCN2 and AQP1 gene were significantly involved in Adenoid Cystic Carcinoma (ACC) in the mouse models[36,37]. Similar studies are required in the humans with a focus on identifying the epigenetic signatures in body fluids and pathological tissues. There are few studies which have demonstrated that the body fluids such as saliva, blood (isolated T lymphocytes) can have overlapping epigenetic signatures with pathological tissues, thus may serve as non-invasive biomarkers especially where biopsy is not possible [38,39].

3.2 Histone Modifications in Oral Cancer

There are only a few number studies available reporting histone modifications in OSCC. Most of these studies have depicted histone methylation of H3 histone in OSCC at various

Lysine molecule positions [k27=45 %, k9=47% and k4=39% of oral cancers samples analyzed]. In contrast to that acetylation of H3 histone was also observed at specific lysine molecule positions (k4=37%, k9=80%, k18=39% of oral cancers samples analyzed). The percentages/ratios for lysine molecule positions were calculated by dividing the total number of methylated samples with the total number of samples being analyzed [40–43]. Furthermore, the correlations were drawn between histone modifications and clinicopathological parameters like nodal status, the size of the tumor, tumor stage, perineural invasion in oral squamous cell carcinoma. H3K27me3, H3K18ac, and H3K4ac histone marks revealed a positive correlation with clinicopathological parameters[41]. In addition, H3K9me3 marks at HOX genes were considerably lower in OSCC as compared to the normal tissue[44].

In ACC, the higher levels of histone H3 lysine 9 trimethylation (H3K9me3) expression has been considered as a predictor of rapid cell proliferation and distant metastasis, thereby poor patient survival[45].

3.3 Micro RNAs and Oral cancer

The role micro RNAs (miRNAs) in oral cancer has been well explored in the past few years. miRNAs play a key role in tumorigenesis of cancer stem cells through mechanisms such as drug resistance, tumorigenicity, and self-renewal[46]. Genome-wide studies have revealed some micro RNAs undergoing upregulation and downregulation in oral cancer lesions.

In a meta-analysis, D Souza et al. reported overexpression of micro RNAs in 70.07% of OSCC lesions, whereas downregulation was observed in 69.5% of samples [47,43]. In 80% of OSCC samples, miR-146a, miR-211, miR-31, miR-21, miR-204, miR-24, and miR-155 were seen most frequently upregulated. Upregulation of these miRNAs was associated with

clinicopathological features like regional metastasis, advanced tumor stage[47,43,48].

Micro RNAs have a dramatic effect on a number of signaling pathways in OSCC. For instance, miR-21 has been shown to promote oral cancer targeting Dickkopf-related protein 2 (DKK2) via Wnt/beta-catenin pathway[49]. Similarly, a recent study has also implicated miR-373-3p to induce metastasis targeting DKK1 via Wnt/beta-catenin pathway[50]. In addition, miR-218 has been shown to increase cisplatin drug resistance via protein phosphatase 2 regulatory subunit B'alpha (PPP2R5A)/Wnt signaling pathway[51]. It has been reported that Wnt1 inducible signaling pathway protein 1 (WISP1), involved in developmental functions may show polymorphisms in betel nut chewing and smoking, thus may serve as a potential biomarker[52].

Similar to OSCC, micro RNAs affect a number of signaling pathways in ACC. In a recent study, miR-155 was proposed to be involved in ACC metastasis affecting ubiquitin-like modifier activating enzyme 2 (UBA2) pathway suggesting UBA2 as an epigenetic biomarker for ACC metastasis[53]. Similarly, miR-21 dysregulation plays an important role in tumor growth and invasion targeting programmed cell death protein 4 (PDCD4) via signal transducer and activator of transcription 3 (STAT3) pathway[54]. Another study demonstrated that miR-155 is involved in the growth and invasion of ACC affecting epidermal growth factor receptor (EGFR)/NF-kappaB pathway[55].

4 Epigenetics and Personalized Medicine:

A significant challenge for clinicians for everyday practice is to develop right diagnosis and provide appropriate treatment for a disease. Clinical research has revealed that there is

variation in the response to treatment among individuals, and sometimes same therapy becomes ineffective in few individuals. This variation in response to treatment has led to the concept of personalized medicine. Personalized medicine can be defined as using the molecular analysis to tailor the best treatment for an individual based on their characteristics. The characteristics of a patient can be grouped into genetic, epigenetic, proteomic, metabolomic and exposome categories. Epigenetic profile of a patient represents an integral and sensitive part of the personalized medicine[66].

In terms of personalized medicine, the epigenetic biomarkers such as DNA methylation-based biomarkers, histone biomarkers, and micro-RNA based biomarkers serve as a potent diagnostic tool to assess response to specific therapy in clinics. Epigenetic factors have a key advantage over genetic factors regarding sensitivity for the diagnosis of a disease. For example, the genetic factors include mutational analyses like single nucleotide polymorphisms (SNPs) and copy number variations (CNVs) concerning their clinical application. In contrast to that, for a given marker gene epigenetic factor like DNA methylation analysis focuses on a small promoter region as compared to a mutational analysis of genetic factors which rather focus on numerous different point mutations throughout the length of the gene. DNA methylation modifications occur at higher percentages than genetic changes in tumors, resulting in higher sensitivity[66].

In epigenetic biomarkers, Foy et al., using high throughput sequencing, studied genome-wide methylation changes in oral premalignant lesions (OPL) in humans. They identified 86 genes which were differentially methylated in OPL as compared to the normal oral mucosa. Furthermore, 86 genes were hypermethylated in developing oral squamous cell carcinoma

(OSCC). Out of 86 genes, they suggested AGTR1, FOX12 and PENK promoter methylation significantly associated with an increased risk of development of oral squamous cell carcinoma in patients with OPL[59]. In another study, the PAX1 gene was considered as a promising biomarker for the detection of OSCC[60]. Similarly, hypermethylation of HOX9 gene was seen frequently in oral cancer with significant risk of metastasis, suggesting HOX9 as a potent biomarker for oral cancer-related metastasis[61]. In addition to that HOX9 and NID2 promoter hypermethylation in OSCC and saliva samples suggested their use as potential biomarkers in early detection of the oral cancerous lesion[62]. Also, KIF1A and EDNRB genes were identified as potential biomarkers in Head and Neck Squamous cell carcinoma[63]. In other words, these DNA methylation-based biomarkers strongly suggest their use for early diagnosis of cancer.

Epigenetic biomarkers like MGMT and MLH1 gene are strongly considered as a predictor of response to chemotherapy in various cancerous lesion[64]. Similar to oral cancer, various response predictor genes can be discovered for their use as potential biomarkers in assessing treatment with respect to chronic periodontal diseases.

A major gap in epigenetic personalized medicine is a lack of merging the molecular analysis data to current clinical practice. A number of validation studies are required before their use as biomarkers in pain and other dental diseases, and thus, no any systematic review or meta-analysis is available except oral cancer.

5 Conclusion:

Epigenetics has opened new doors for clinical and basic science research in the field of dental/oral health research. Epigenetic modifications have been widely studied in various oral pathologies, but their causative factors are less studied especially in periodontal disease. There

is need to explore the effect of epigenetic changes in the pathogenesis of the periodontitis, oral cancer and to understand dysregulated signaling pathways behind them. Since environmental changes or exposure (exposome) significantly impacts the epigenome, the molecular events regulated by exposome should be well studied to understand the development of dental or oral pathologies. By assessing the information from these studies including exposomes, genomes, epigenomes, proteomes, and metabolomes, a personalized medicine approach can be drawn in periodontal diseases as well. The information collected in the form of biomarkers will allow the dentist/clinicians to decide if specific therapy responds to specific pathology in a specific individual.

Ethical Approval

Ethical approval was not required.

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Declaration of Conflicts of Interest:

The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article

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Genome-Wide DNA Methylation Studies in Oral Squamous Cell Carcinoma (OSCC)					
Author/Year	Species	Methods for genome-wide profiling	Methods used to verify candidate genes	Verified Candidate Genes	Summary
Sun et al. 2017[65]	Mice	ERRBS	RT-PCR	**Hypermethylated genes**: Pkm, Ppp1r13I, Vamp3, Ctnnb1, and Tbc1d4 **Hypomethylated genes**: Ppp1r21, C1qtnf9, Fgf3, Efemp2	Fgf3 gene hypomethylation is significantly associated in OSCC, thereby offering its use in early detection of OSCC as an epigenetic biomarker.
Khongsti et al. 2017[66]	Human	Infinium 450 K methylation assay	RT-PCR	Wt1	Wt1 genes showed hypermethylation at promoter region and decreased mRNA expression. In total, 45 genes with DMRs at promotor region were observed. Hypermethylation of 38 genes and hypomethylation of 7 genes was observed.
Basu et al 2017[67]	Human	Infinium 450 K methylation assay	Bisulfite sequencing PCR and RT-PCR	Hypermethylated genes: HLA-DPB1, LDLRAD4, LHX1, and LXN Hypomethylated genes: PTPN22	Hypomethylation of immune genes was observed. Pathway analysis of these genes revealed that anti-tumor T cell response may play a role in the mobilization of lymphocytes at tumor microenvironment.
Bhat et al. 2017[68]	Human	DMH Microarray	Bisulfite sequencing PCR	Hypermethylated genes: DAPK1, LRPPRC, RAB6C, and ZNF471	Hypermethylation of LRPPRC, ZNF471, RAB6C in OSCC offers diagnostic and prognostic potentials in head and neck squamous cell carcinoma.
Lim et al. 2016[69]	Human	Infinium 450 K methylation assay	-	-	The study was unable to find any prognostic methylation profiles.
Clausen et al. 2016[70]	Human	MethylCap-Seq	Bisulfite sequencing PCR	WISP1	Hypomethylation of WISP1 offers the diagnostic potential to serve as an epigenetic biomarker to predict lymph node metastasis.
Krishnan et al. 2016[71]	Human	Infinium 450 K methylation assay	qMSP, RT-PCR	Hypermethylation of MIR10B gene. Other differentially methylated genes: FUT3, TRIM5, TSPAN7, MAP3K8, RPS6KA2, SLC9A9, and NPAS3	Hypermethylation of MIR10B gene was consistent with the differential expression of its target genes NR4A3, BCL2L11 thus suggesting NR4A3 as a potential target for therapeutic intervention to treat OSCC.
Khor et al. 2014[72]	Human	Infinium 450 K methylation assay	Bisulfite sequencing PCR	Differential hypermethylation of TP73, PIK3R5, and CELSR3 genes	Signature candidates can offer the potential to use as epigenetic biomarkers in OSCC.
Lai et al. 2014[73]	Mice	Microarray Hybridization	Bisulfite sequencing PCR, RT-PCR	Hypermethylation of RARB	RARB hypermethylation and de novo DNMTs are involved in the areca-associated oral cancer.
Marcinkiewicz et al. 2014[44]	Human	ERRBS	-	-	H3K9me3 marks at HOX genes were considerably lower in OSCC. Both histone modifications and altered DNA methylation pattern are involved in dysregulation of homeobox gene expression in OSCC.
Zhang et al. 2013[74]	Human	MeDIP and methylation microarray hybridization.	Bisulfite sequencing PCR, RT-PCR	Hypermethylation and decreased expression of FBLN1, ITIH5. Hypomethylation and increased expression of RUNX3	Aberrant DNA Methylation plays an important role in OSCC
Genome-Wide DNA Methylation Studies in Adenoid Cystic Carcinoma (ACC)					
Author/Year	Species	Methods used for genome-wide profiling	Methods used to verify candidate genes	Verified Candidate Genes	Summary
Ling et al. 2016[37]	Mice	Infinium 450 K methylation assay	Bisulfite sequencing PCR	HCN2	HCN2 hypomethylation can be used as an epigenetic biomarker to detect ACC.
Shao et al. 2011[36]	Mice	Global demethylation+ expression microarray	qMSP, RT-PCR	AQP1	Hypomethylation and overexpression of AQP1 can be used as an epigenetic biomarker to detect ACC

Abbreviations used: ERRBS (Enhanced Reduced Representation Bisulfite Sequencing, DMH Microarray (Differential Methylation Hybridization Microarray), qMSP (Quantitative Methylation Specific PCR), MeDIP (Methylated DNA Immunoprecipitation)

Table 1

Figure Legend

Figure 1) A schematic representation of the epigenetic mechanisms occurring at cellular and molecular level. 1) DNA Methylation 2) micro RNA based mechanisms 3) Histone Modifications. Acronyms used: HDACs (Histones deAcetylases), DNMTs (DNA MethylTransferases), Ub (Ubiquitination), DUBs (Deubiquitinating enzymes), miRNA (microRNA), RNA Polymerase II (RNA pol II), primary miRNA (pri-miRNA), precursor miRNA (pre-miRNA), RNA-induced silencing complex (RISC), DiGeorge Syndrome Critical Region 8 (DGCR8)