DNA Methylation for Head and Neck Cancer

Subjects: Oncology

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Head and neck squamous cell carcinoma (HNSCC) is a term collectively used to describe all cancers that develop in the oral and nasal cavities, the paranasal sinuses, the salivary glands, the pharynx, and the larynx. A biomarker is a biological finding that stands in for and optimally forecasts a clinically related outcome or an intermediate result that is more difficult to detect. It is a specific characteristic that is measured as an indicator of the normal biological procedures, pathological mechanisms or responses to an exposure or interference. Recent evidence suggests that DNA methylation can alter the expression of genes in a way that it favors tumorigenesis and tumor progression in HNSCC, and therefore represents a potential source for biomarker identification.

Keywords: head and neck cancer ; DNA methylation ; diagnostic biomarkers

1. Introduction

Head and neck squamous cell carcinoma (HNSCC) is a general term that includes all cancers that develop in the oral and nasal cavities, the paranasal sinuses, the salivary glands, the pharynx, and the larynx [1]. Most of these cancers are initiated in the squamous cells that line the moist surfaces inside the head and neck (hence the term head and neck squamous cell carcinoma), and are characterized by heterogeneity in their phenotypic, clinical, and biological features [2][3][4][5]. According to the Globocan online database, in 2020, it was estimated that there were 931,931 new cases of HNSCC and 467,125 related deaths worldwide [6]. The majority of patients who will be diagnosed at an early stage can be cured [4]. However, patients with locally advanced and aggressive disease, who account for 75% of the newly diagnosed cases, are likely to experience relapse and have a 5-year overall survival (OS) rate of 50%, despite advances in surgical treatment, radiotherapy, and chemotherapy [4][5]. Consequently, there is a need for the identification and application of tools with high sensitivity and specificity that would enable diagnosis at the earliest possible stage, inform clinicians of the possible prognosis of patients, contribute to the early detection of relapses, and provide information on the progression of the disease after the application of specific treatments [7].

2. DNA Methylation Biomarkers with Diagnostic Potential

A diagnostic biomarker is used to identify or verify the occurrence of a disease or a situation of interest, or to designate a specific disease subtype [8].

MicroRNAs (miRNAs) represent small, non-coding RNAs that are involved in gene expression either through repression of translation or through direct degradation of the target mRNA and are also actively implicated in oncogenesis [9]. The methylation status of three promoter sites of microRNA 9 (miRNA 9), namely miR-9-1, miR-9-2, and miR-9-3, are perhaps the most widely studied microRNAs in patients with HNSCC [9]. Oral and oropharyngeal carcinomas are characterized by higher miR-9-1 and miR-9-3 methylation levels, as compared to laryngeal carcinomas, while miR-9 expression appears to be reduced [9]. Consequently, the methylation patterns of miR-9-1 and miR-9-3 are regarded as sensitive and highly specific for the diagnosis of HNSCC, especially oral and oropharyngeal carcinomas [9].

The *EDNRB* gene encodes the B-type endothelin receptor (G protein-coupled receptor) that activates a phosphatidylinositol-calcium second messenger system, while *DCC*, a tumor suppressor gene, encodes a transmembrane protein with structural homology to NCAM, which is involved in the differentiation of epithelial and neuronal cells [10]. The promoters of both genes have been found to be hypermethylated in 40% of saliva samples from patients with oral cavity cancer (OCC) and precancerous lesions [10]. Thus, hypermethylation of *DCC* and *EDNRB* has been associated with malignant histopathological diagnosis, independent of other factors such as age, smoking, and alcohol consumption; this further suggests that these genes can be used as individual biomarkers of malignant transformation for the screening of high-risk patients, as well as for the identification of patients who might appear to be at low risk during physical examination, but are categorized as high risk based on the salivary methylation biomarkers [10].

The mediator complex subunit 15 gene (*MED15/PCQAP*) encodes a cofactor that contributes greatly to the modulation of transcription initiation in the promoters of many genes [11]. Two CpG dinucleotide clusters (at the 5′ or 3′ end) of this gene have been identified to be methylated in HNSCC tumors in patients who have been smokers, but not in normal tissue samples derived from the same subjects, with subsequent validation of the findings in saliva samples [11]. It has therefore been concluded that these CpG methylation sites may be used as potential non-invasive biomarkers for HNSCC detection [11]. Specifically, the 5′-CpG site has emerged as a stronger diagnostic biomarker than the 3′-CpG site, with the DNA methylation levels of both sites being comparatively lower in saliva samples of HPV+ patients as compared to HPV− patients [11]. Moreover, it has been reported that the differential methylation of *ZNF14*, *ZNF160,* and *ZNF420*, all of which are members of the zinc finger gene family, constitutes an important biomarker of HNSCC identification, exhibiting 100 % specificity in primary tissue and saliva samples [12].

Other genes with abnormal methylation patterns and consequently with differential expression patterns in HNSCC tissues have been detected through bioinformatics analysis [13]. Two hypermethylated genes with concomitant low expression, *FAM135B* and *ZNF610*, and two hypomethylated genes with concomitant high expression, *HOXA9* and *DCC*, have been identified, and their diagnostic utility has been validated through ROC curve analysis [13]. In contrast to these observations, the *HOXA9* promoter appears to be characterized by considerably higher methylation levels in pathological tissues, as compared to normal controls, and may therefore serve as an early diagnostic biomarker in patients with HNSCC [14]. Nonetheless, a statistically significant difference in *HOXA9* methylation seems to exist between men and women with HNSCC [14].

In HPV+ oropharyngeal squamous cell carcinoma (OPSCC), 20 differentially methylated DNA regions (DMRs) have been identified in the following genes: *KCNA3*, *EMBP1*, *CCDC181*, *DPP4*, *ITGA4*, *BEND4*, *CTNND2*, *ELMO1*, *SFMBT2*, *C1QL3*, *MIR129-2*, *ATP5EP2*, *OR6S1*, *NID2*, *HOXB4*, *ZNF439*, *ZNF93*, *VSTM2B*, *ZNF137P*, and *ZNF773* [15]. The methylation levels in HPV+ OPSCC are remarkably higher than those in normal samples and non-HPV-related HNSCC, and as such, these 20 DMRs have been suggested as potential diagnostic biomarkers in patients with HPV+ OPSCC [15].

Prominin 1 (*PROM1*) encodes a pentaspan membrane glycoprotein, often expressed on adult stem cells [16]. The *PROM1* promoter appears to be hypermethylated in HNSCC tissues, as compared to normal head and neck tissues, while increasing methylation levels are negatively associated with *PROM1* gene expression, with the highest methylation levels observed in smokers and elderly patients [16]. Overall, the methylation status of *PROM1* may serve as a valuable biomarker for the early diagnosis of HNSCC [16].

The collagen triple helix repeat containing 1 (*CTHRC1*) gene encodes an extracellular matrix protein that acts as a modulator of the tumor microenvironment, and appears to be overexpressed in HNSCC tissues, as compared to healthy tissues, due to promoter hypomethylation [17]. Furthermore, plasminogen activator urokinase (PLAU) overexpression may be an independent diagnostic and prognostic biomarker in HNSCC [18]. PLAU is a protease involved in numerous different signaling pathways, including apoptosis, epithelial-mesenchymal transition (EMT), and Ras/MAPK; hypomethylation of *PLAU* gene as well as hypomethylation and subsequent downregulation of miR-23b-3p, a microRNA that targets *PLAU*, may be responsible for the overexpression and the oncogenic role of *PLAU* in HNSCC [18].

Other genes with potential diagnostic utility in HNSCC include methylenetetrahydrofolate dehydrogenase 1 (*MTHFD1L*), the opioid receptor genes *OPRL1* (opioid-related nociceptin receptor 1) and *OPRM1* (opioid receptor mu 1), and the xenotropic and multimodal retrovirus receptor 1 (*XPR1*). *MTHFD1L* expression levels have been found to be significantly higher in 24 subtypes of HNSCC, compared to normal controls, and to be accompanied by promoter hypomethylation [19]. Similarly, in plasma liquid biopsy samples from patients with OCC, *OPRL1* and *OPRM1* genes appear to be highly methylated as compared to normal tissue derived from the same patients [20]. Last but not least, XPR1, a cell surface receptor for certain types of murine leukemia viruses, exhibits markedly increased expression in HNSCC tissues, as compared to healthy controls, while promoter methylation is significantly lower than that in healthy controls [21].

Table 1 includes a list of DNA methylation biomarkers with diagnostic potential [9][10][11][12][13][14][15][16][17][18][19][20][21].

Table 1. Diagnostic and Screening biomarkers.

References	Cancer Type	Biomarker Name-Gene	Function	Sample Type	Biomarker Behavior	Application
[9]	Oral and oropharyngeal	MiR-9	Non-coding RNA implicated in the modulation of gene expression, tumor suppression through inhibition of cellular pro-proliferation and modulation of PTEN.	Tissue	Promoter hypermethylation/sites 1 and 3.Low expression.	Diagnosis
[10]	Oral	EDNRB	It encodes the B-type endothelin receptor (G protein-coupled receptor) that triggers a phosphatidylinositol-calcium second messenger system.	Saliva	Promoter hypermethylation in 40% of malignant samples.	Screening, Diagnosis
[10]	Oral	DCC	Tumor suppressor, encodes a transmembrane protein with structural homology to NCAM, which is associated with the differentiation of epithelial and neuronal cells.	Saliva	Promoter hypermethylation in 40% of malignant samples.	Screening, Diagnosis
[11]	HNSCC	MED15/ PCQAP	Encodes a cofactor with pleiotropic activity, important for the formation of the RNA polymerase II complex, which is involved in the expression of all protein-coding genes, attenuation of at least one of the signaling pathways (TGFβ/Activin signaling).	Tissue, saliva	Specific methylation patterns in the promoter.	Screening, Diagnosis
[12]	HNSCC	ZNF14, ZNF160, ZNF420	Members of the zinc finger gene family.	Tissue, saliva	Differential methylation pattern.	Diagnosis
[13]	HNSCC	FAM135B	Involved in the maintenance of the nucleolus, cell proliferation, cell differentiation, and apoptosis.	Tissue	Hypermethylation. Low expression.	Diagnosis
[13]	HNSCC	ZNF610	Involved in the maintenance of the nucleolus, cell proliferation, cell differentiation, and apoptosis.	Tissue	Hypermethylation. Low expression.	Diagnosis
[13]	HNSCC	HOXA9	Involved in the maintenance of the nucleolus, cell proliferation, cell differentiation, and apoptosis.	Tissue	Hypomethylation. High expression.	Diagnosis
[13]	HNSCC	DCC	Involved in the maintenance of the nucleolus, cell proliferation, cell differentiation, and apoptosis.	Tissue	Hypomethylation. High expression.	Diagnosis

References	Cancer Type	Biomarker Name-Gene	Function	Sample Type	Biomarker Behavior	Application
[14]	HNSCC	HOXA9	Encodes a transcription factor. HOX genes regulate and specify different cell types during embryonic growth, and have important functions in the modulation of the sensitive balance between cell proliferation and differentiation during cancer development.	Tissue	Promoter hypermethylation.	Screening, Diagnosis

References	Cancer Type	Biomarker Name-Gene	Function	Sample Type	Biomarker Behavior	Application
[15]	HPV+ OPSCC	KCNA3, EMBP1, CCDC181, DPP4, ITGA4, BEND4, CTNND2, ELMO1, SFMBT2, C1QL3, MIR129-2, ATP5EP2, OR6S1, NID2, HOXB4, ZNF439, ZNF93, VSTM2B, ZNF137P and ZNF773	ITGA4: on the cell surface, promotes migration and adhesion to the microenvironment in chronic lymphocytic leukemia. NID2: component of the basement membrane that stabilizes the extracellular matrix (ECM) network. Suppresses migration and blocks metastasis by downregulating the EGFR/AKT and integrin/FAK/PLCγ pathways. HOXB4: a hematopoietic transcription factor, downregulates the expression of Prdm16, which is a proto-oncogene necessary for self-renewal and preservation of mouse hematopoietic stem cells. SFMBT2: tumor suppressor, negatively regulates migration and invasion by targeting MMP-9 and MMP-26. MIR129-2: tumor suppressor, inhibits migration and invasion by directly inhibiting HMGB1. DPP4 and CTNND2: act both as tumor suppressors and as markers of tumor invasiveness, depending on the type of tumor. KCNA3: its knockdown markedly suppresses cell proliferation and increases apoptosis. EMBP1: related to ER-positive breast cancer and lower grade breast tumors. ZNF93: implicated in the DNA repair pathway following DNA damage by chemotherapy. Little is known about six candidates (ATP5EP2, OR6S1, ZNF439, VSTM2B, ZNF137P, ZNF773). ZNF439, ZNF137P, ZNF773: belong to the zinc finger protein group, members of which have previously been shown to have tumor-suppressive activity.	Tissue	20 differentially DMRs → hypermethylation.	Screening

References	Cancer Type	Biomarker Name-Gene	Function	Sample Type	Biomarker Behavior	Application
[16]	HNSCC	PROM1/CD133	Encodes a pentaspan membrane glycoprotein, which is frequently found in adult stem cells.	Tissue	Promoter hypermethylation. Low expression.	Diagnosis
[17]	HNSCC	CTHRC1	Encodes an extracellular matrix protein, modulating tumor microenvironment	Tissue	Promoter hypomethylation. High expression.	Diagnosis
[18]	HNSCC	PLAU	Encodes a protease implicated in apoptosis, epithelial-mesenchymal transition (EMT), and the Ras/MAPK pathway.	Tissue	Hypomethylation. High expression.	Diagnosis
[19]	HNSCC	MTHFD1L	Encodes a cytoplasmic enzyme participating in the formation of tetrahydrofolic acid (THF) within mitochondria.	Tissue	Promoter hypomethylation. High expression.	Diagnosis
[20]	HNSCC	Opioid receptor mu 1(OPRM1)	Encodes an opioid receptor (G-protein-coupled receptor).	Tissue, plasma	Hypermethylation.	Screening
[20]	HNSCC	Opioid-related nociceptin receptor 1(OPRL1)	Encodes an opioid receptor (G-protein-coupled receptor).	Tissue, plasma	Hypermethylation.	Screening
[21]	HNSCC	XPR1	Encodes a cell surface receptor for certain types of murine leukemia viruses, which plays an essential role in maintaining intracellular phosphate homeostasis by mediating phosphate export from the cell.	Tissue	Promoter hypomethylation. High expression.	Diagnosis

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