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REVIEW ARTICLE

DNA methylation in tumour and normal mucosal tissue of head and neck squamous cell carcinoma (HNSCC) patients: new diagnostic approaches and treatment

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Abstract Head and neck squamous cell carcinoma (HNSCC) is the sixth most common malignancy worldwide. Long-term survival of this patient group has been marginally improved during the last 30 years. This is due to the high recurrence rate of local primary or development of second primary tumours in the patients. We found that normal-appearing surgical margins and distant mucosal tissue of HNSCC patients contained tumour suppressor genes DNA methylation. These cells might be the progenitors of the tumour recurrences. Such molecular abnormalities in the normal-appearing mucosa tissue were not possible to detect in the clinic or by standard histopathologically analysis. To improve clinical outcome, the convenient and cost-effective molecular analysis such as methylation-specific PCR should be added to the pathological diagnosis armamentarium for HNSCC patients. The beneficial effect of antimethylating agents as additional treatment or for cancer chemoprevention, in this high-risk patient group, warrants further investigation.

Keywords Head and neck squamous cell carcinoma (HNSCC) · Epigenetic · DNA methylation

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Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common malignancy worldwide [1]. The first degree relatives of the HNSCC patients were shown to have higher risk of developing cancer [2]. Epidemiological data established that cigarette smoke and high alcohol consumption are risk factors for this type of cancer [3, 4]. Persistence of smoking and drinking after treatment is strongly related to the development of a second primary tumour [5]. Despite the advances in therapy, long-term survival of the patient has not improved significantly over the last 20 years [6]. The lack of progress is due to the relatively high recurrence rates of tumour observed in these HNSCC patients [7].

Incidence of local tumour recurrences in HNSCC was suggested to be related to either field cancerisation or clonally expansion [8, 9]. According to the field cancerisation model, multiple HNSCC arise from separate or independent cell clones. Alternatively, one clone from a single progenitor cell gives rise to primary tumour and its daughter cells to metastasis [10]. Regardless of their origin, the recurrences of primary or second primary HNSCC are the key factors in the treatment and the clinical outcome of these patients.

The purpose of this article is to present current information regarding DNA methylation for diagnosis and possible prevention of tumour recurrence in HNSCC patients.

Effects of cigarette smoke and alcohol on normal cells

From the initiation of carcinogenesis, normal cells must proceed through various changes prior to becoming

malignant. Epithelial cells in the head and neck are the first to be exposed to various carcinogenic agents from cigarette smoking and drinking. We recently demonstrated that cigarette smoke in combination with alcohol strongly induced massive cell death and various abnormalities in the surviving normal cells [11]. Long-term exposure to cigarette smoke and alcohol will induce both chronic inflammation from massive cell death and the outgrowth of abnormal cells within an inflammatory microenvironment. Expansion of these precancerous cells within the non-tumour tissue phenotype in the head and neck area could be expected [12].

DNA methylation and HNSCC

DNA methylation is an integral epigenetic component of cellular development and differentiation as well as a basis of human diseases. Transfer of a methyl group exclusively at C-5 position of cytosine base, located in cytosine-guanosine dinucleotides (CpGs), will modify the structure of DNA without changing the DNA sequence [13]. DNA methylation determines cell fate, phenotype and plays an important role in the regulation of gene expression. The so-called CpGs island, located within the promoter regions of genes, is generally unmethylated in normal cells. Mostly, human cancer consists of aberrantly genomewide global hypomethylation and local hypermethylation at some CpGs island [14].

While the genetic code in an individual is stable and similar in every cell, epigenetic code could be tissue specific or cell specific and could be changed according to its environmental situation [15]. It is important to note that DNA methylation is reversible and more frequently observed than genetic mutations in various types of cancer including HNSCC [16].

Abnormal methylation of CpGs regions in the tumour suppressor genes leads to silencing of tumour suppressor functions, central to the development of solid tumours [17]. DNA hypomethylation in the CpGs island of an oncogene will have an increased potential for gene activity and chromosome instability [18]. Thus, DNA methylation will have a direct impact on both the mutational and the epigenetic components of neoplastic transformation.

Several methods of determination of DNA methylation have been described including the use of restriction enzymes, microarray-based methylation analysis, genomic bisulphite sequencing and methylation-specific PCR (MSP). Of these methods, MSP analysis is the most convenient, rapid and cost effective [19].

DNA methylation was observed in HNSCC and the premalignant tissue in HNSCC patients [20]. Using MSP analysis, distant or surrounding normal-appearing mucosal tissues of HNSCC patients contained abnormal cells with various tumour suppressor genes methylations [21]. Abnormalities in the tumour suppressor genes might allow uncontrolled cell cycle and division as indicated by abnormal DNA content, aneuploidy and tumour progression [21, 22]. DNA methylation of multiple tumour suppressor genes was suggested to be associated with shortened survival after standard therapy in HNSCC patients [23, 24].

Based on DNA methylation, precancerous cells and cancerous cells are more widespread than previously recognised in the clinic [21, 25]. Regrettably, it is not feasible to remove all the suspicious areas in the head and neck region of the patients by excise or radiation. Alternative means of therapy or prevention with chemical compounds that can affect the entire head and neck region could benefit to these patient group [26].

Many epigenetic changes, including DNA methylation, are reversible. These normal mucosal phenotype tissues with epigenetic alteration could be controlled by reversal of the cancerous process or halted in their cancerous process with demethylation agents [27, 28]. It has been reported that reversal of epigenetic silencing by demethylation agents enhances the effects of radiotherapy [29, 30]. This suggests that demethylation agent might be used as additional treatment for the HNSCC patients who are at greatest risk of local tumour recurrences or developing second primary tumours after surgery or radiotherapy [31, 32]. Thus, the possibility of demethylation agents as therapeutic intervention or cancer chemoprevention could be worth further investigation.

Conclusion

High alcohol consumption and cigarette smoking might induce a widespread area of the precancerous cells with epigenetic change and chronic inflammatory microenvironment in the head and neck region [33, 34]. These abnormal tumour progenitor cells within the normal-appearing margins and distant mucosal tissues are beyond visibility in the clinical and pathology macroscopically or microscopically examination [21, 35]. To detect such change, an additional molecular analysis is needed.

We propose that MSP analysis could be used as a molecular diagnostic marker adjunct to histopathological examination for detection of the precancerous tissue or metastasis tumour cells in HNSCC patients. MSP analysis is robotic, rapid and requires only a common machine, available in all routine laboratories. MSP's analysis is also cost effective and affordable within a general clinical setting. For improving outcome of HNSCC patients, DNA methylation analysis and methylation-targeted therapies should be investigated in a clinical trial of HNSCC patients.

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Conflict of interest None of the authors have any actual or potential conflict of interest, including any financial, personal or other relationships with people or organisation that could inappropriately influence this work.

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