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Mini-review

Delineating an epigenetic continuum in head and neck cancer

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ABSTRACT

A tissue field of somatic genetic alterations precedes the histopathological phenotypic changes of carcinoma. Genomic changes could be of potential use in the diagnosis and prognosis of pre-invasive squamous head and neck carcinoma (HNSCC) lesions and as markers for cancer risk assessment. Studies of sequential molecular alterations and genetic progression of pre-invasive HNSCC have not been clearly defined. Studies have shown recurring alterations at chromosome 9p21 (location of the *CDKN2A*) and *TP53* mutations in the early stages of HNSCC. However, gene silencing via hypermethylation is still a relatively new idea in the development of HNSCC and little is known about the contribution of epigenetics to the development of neoplasia, its transformation, progression, and recurrence in HNSCC. This review examines the role of promoter hypermethylation of tumor suppressor genes in the progression continuum from benign papillomas to malignancy in HNSCC.

1. Introduction

Head and neck squamous cell carcinoma (HNSCC) is one of the most prevalent cancers in the world with over 500,000 cases diagnosed annually. In the United States, approximately 52,140 new cases are expected in 2011 with an estimated 11,460 deaths for HNC of the oral cavity, pharynx, and larynx [1].

Despite considerable efforts, the 5-year survival rate for HNSCC has not changed significantly making accurate and reliable stratification for prediction of outcomes challenging. Much of this is attributed to the numerous anatomic sites and subsites from which tumors can arise and the diversity of histologic types of tumors in these locations [2]. Patients with advanced HNSCC are limited to a complete response of 50% and often require long-term rehabilitation. However, early HNSCC detection increases survival to 80%. To facilitate timely diagnosis and improve treatment, elucidation of early detection markers is crucial. A current shortcoming in the prognosis and treatment of HNSCC is a lack of methods and large study cohorts to adequately address the etiologic complexity and diversity of the disease.

1.1. Genomic advances in HNSCC

Cancer is the result of transformation from a normal to a malignant cell that results from accumulated mutations. Acquisition of a fully malignant phenotype in colon cancer is thought to occur as a result of multiple steps whose targets are alterations of growth-promoting oncogenes and growth-inhibiting cancer suppressor genes [3]. The evolution in transformation from a normal squamous epithelial cell to a cancer cell is likewise assumed to require several steps, some defined by genetic alteration. However, the precursor lesion(s) and sequence of events are less clearly defined for head and neck squamous cell carcinoma (HNSCC).

1.2. Genetics of HNSCC

Early cytogenetic studies of HNSCC relied on analysis of later stage tumors and established cell lines. Recent short-term cell cultures have indicated similar genetic changes. Common sequences of SCC karyotype evolution appear to require initial loss of chromosome segments, followed by tetraploidization, and ultimately loss of previously uninvolved chromosomes from the tetraploid population [4–6]. A universal class of cytogenetic change is deletions, also observed as loss of heterozygosity (LOH). LOH /microsatellite instability at 3p, 9p, 17p, and 18q chromosomal locations [7] are among the most common [5,6,8–11]. Patients with benign premalignant lesions that harbored HNSCC specific genetic loses and LOH had a significantly increased risk of developing cancer [12].

Mutations in the tumor suppressor *p53* gene occur in 45–70% of HNSCC and strategies targeting the *p53* gene and protein may halt or reverse the process of tumorigenesis [13]. Another important gene product in HNSCC pathogenesis is the $p16^{INK4a}$ (p16) protein made by the $p16^{INK4a}$ (*CDKN2A*) gene located at 9p21. p16 is a cyclin-dependent kinase inhibitor that inhibits phosphorylation of

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the retinoblastoma protein (pRb) and blocks cell cycle progression at the G1 to S check point [14]. Loss of p16 expression by deletion, mutation, or hypermethylation is common in HNSCC [15,16] and is associated with worse prognosis in laryngeal squamous cell carcinomas [17].

1.3. Epigenomics and cancer

The study of human disease has focused primarily on genetic mechanisms. Dispelling the belief that the only way to treat such conditions is by fixing or replacing damaged genes, scientists are instead focusing on the field of epigenetics – the study of changes in gene silencing that occur without changing the DNA sequence. Many types of epigenetic processes have been identified – they include methylation, acetylation, phosphorylation, ubiquitylation, and sumolyation. Epigenetic processes are natural and essential to many organism functions, but if they occur improperly, there can be major adverse health and behavioral effects.

Perhaps the best known epigenetic process, in part because it has been easiest to study with existing technology, is DNA methylation. This is the addition or removal of a methyl group (CH_3). Hypermethylation is a well described DNA modification that has been implicated in normal mammalian development [18,19], imprinting [20] and X chromosome inactivation [21]. However, recent studies have identified hypermethylation as a probable cause in the development of various cancers [22–24]. Aberrant methylation by DNA-methyltransferases in the CpG islands of a gene's promoter region can lead to transcriptional repression akin to other abnormalities such as a point mutation or deletion [25]. Gene transcriptional inactivation via hypermethylation at the CpG islands within the promoter regions is an important mechanism [26]. This anomalous hypermethylation has been noted in a variety of tumor-suppressor genes (TSGs), whose inactivation can lead many cells down the tumorigenesis continuum [26–28]. In many cancers, aberrant DNA methylation of so called "CpG islands", CpG-rich sequences frequently associated with promoters or first exons, is associated with the inappropriate transcriptional silencing of critical genes [29–31]. These DNA methylation events represent an important tumor-specific marker occurring early in tumor progression and one that can be easily detected by PCR based methods in a manner that is minimally invasive to the patient.

1.4. Significance of DNA methylation

When compared to the genomic, which is identical in every cell and tissue in the human body, the epigenome is highly variable over the life course, from tissue to tissue and from environment to environment [32]. Also, unlike genes that are inactivated by nucleotide sequence variation, genes silenced by epigenetic mechanisms are still intact and, thus, retain the potential to be reactivated by environmental or medical intervention [32]. There are several current human therapeutic intervention trials to reverse deleterious epigenetic changes. Some examples include epigenetic therapeutic trials to treat T-cell lymphoma based on reactivation of tumor suppressor genes [33] and similar trials to prevent colorectal cancer by inhibiting the enzyme responsible for DNA methylation [34]. Such therapies have shown promise in halting tumor growth by reactivation of the tumor suppressor gene or by blocking progression of precancerous epigenetic lesions. Additionally, demethylating drugs in combination with therapeutic HPV DNA vaccines have been found to control more effectively a variety of HPV-associated malignancies [35]. This is due to the fact that DNA methylation is capable of decreasing expression of the encoded antigen of the DNA vaccines [35]. In fact, preliminary studies already suggest that there is promise of improving preventative HPV DNA vaccine therapy by the addition of the demethylating drug 5-aza-2′deoxycytidine [35].

1.5. DNA methylation in HNSCC

Promotor hypermethylation of genes in HNSCC have been reported for *p16*, *p14*, *DAP-K*, *RASSF1A* [36–42], *RARß2* [43–45], *MGMT*, a DNA repair gene that functions to remove mutagenic (O^6-guanine) adducts from DNA [46], and *E-cadherin*, a Ca^{2+}-dependent cell adhesion molecule that functions in cell–cell adhesion, cell polarity, and morphogenesis [47].

Historically, the molecular pathogenesis of cancer has been teased out one gene at a time. The majority of published epigenetic data in HNSCC comes from methylation specific PCR (MSP) following bisulfite treatment, first described by Herman et al. [48] (gel electrophoresis separation of products). The success of MSP has been attributed to its increased sensitivity, however, it generally relies on a pre-selected number of genes, assessed one gene at a time, as opposed to high-throughput microarray based methylation analysis [49] and multi-candidate gene applications [50]. In HNSCC, recent comprehensive high-throughput methods from our group and others have underscored the contribution of both genetic [15,51,52] and epigenetic events [42,53–57], often working together [50], in the development and progression of HNSCC.

2. Delineating an epigenetic continuum in HNSCC

Gene silencing via hypermethylation is still a relatively new idea in the development of HNSCC. To assess the contribution of epigenetics to the development of neoplasia, its transformation, progression, and recurrence in HNSCC, we examined promoter hypermethylation of tumor suppressor genes along a progression continuum from benign papillomas to malignancy in HNSCC using a multi-candidate gene (Table 1) assay, the Methylation Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA) assay (MS-MLPA, Fig. 1) [50,58]. The candidate gene panel comprises 22 tumor suppressor genes (Table 1), many of which are involved in head and neck cancer.

2.1. Benign papillomas

Papillomas are benign neoplasms of epithelium on a connective tissue core [59]. They can involve the nose and sinuses (sinonasal papillomas – SP) as well as the respiratory tract (respiratory papillomatosis – RP) to include the larynx, trachea, and bronchi. Both SP and RP have a tendency to recur. Recurrent respiratory (laryngeal) papillomatosis (RRP) is an extremely rare condition [60]. Inverted SP are associated with invasive squamous cell carcinoma (SCC) [61] and a small percentage of RRP cases also progress to malignancy [62].

Human papilloma virus (HPV) is frequently associated with sinonasal [63,64] and laryngeal [65–67] papillomas. Most HPV-positive cases of SP are of the inverted type [68]. Benign papillomas are preferentially associated with the low-risk HPV types 6 and 11, whereas their malignant counterparts are exclusively positive for HPV-16 DNA [69]. Studies on HPV typing in benign laryngeal papillomas have demonstrated an association of HPV-11 with a more aggressive course of the disease [70,71]. HPV infection in inverted papillomas [72] and in particular HPV-11 infection in RRP [73] may be an early event in a multistep process of malignant transformation.

2.1.1. Sinonasal papillomas

Sinonasal papillomas have been categorized histologically as inverted, fungiform (exophytic), and cylindrical cell papillomas [74].