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Identification of differentially expressed genes in HPV-positive and HPV-negative oropharyngeal squamous cell carcinomas ☆

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ABSTRACT

Human papillomaviruses (HPVs) have been implicated in the pathogenesis of a subset of squamous cell carcinoma of the head and neck (SCCHN). The goal of this study was to compare the cellular gene expression profiles of HPV-positive and HPV-negative oropharyngeal carcinomas with those of the normal oral epithelium. Using Affymetrix Human U133A GeneChip, our results showed that 397 genes were differentially expressed in HPV-positive SCCHN compared to the normal oral epithelium. The upregulated genes included those involved in cell cycle regulation (*CDKN2A*), cell differentiation (*SFRP4*) and DNA repair (*RAD51AP1*), while the downregulated genes included those involved in proteolysis (*PRSS3*). We also found 162 differentially expressed genes in HPV-negative SCCHN compared to the normal oral mucosa. The upregulated genes included those involved in cell proliferation (*AKR1C3*) and transcription regulation (*SNAPC1*), while downregulated genes included those involved in apoptosis (*CLU*) and RNA processing (*RBM3*). Our studies also identified a subgroup of 59 differentially expressed genes in HPV-positive SCCHN as compared to both HPV-negative SCCHN and normal oral tissues. Such upregulated genes included those involved in nuclear structure and meiosis (*SYCP2*), DNA repair (*RFC5*), and transcription regulation (*ZNF238*). Genes involved in proteolysis (*KLK8*) and signal transduction (*CRABP2*) were found to be downregulated in HPV-positive SCCHN. The results of GeneChip experiments were validated by quantitative real-time RT-PCR analysis of a few representative genes. Our results reveal specific gene expression patterns in HPV-positive and HPV-negative oropharyngeal squamous carcinomas that may serve as potential biomarkers for the development of SCCHN.

☆ Novelty and impact of the paper: Using a 22,200 human transcript oligonucleotide microarray platform, we have examined the gene expression profiles in HPV-positive and HPV-negative oropharyngeal squamous cell carcinomas as compared to the normal oropharyngeal mucosa. Our study has identified several new genes whose expression is specifically associated with the presence of HPV-16 in the oropharyngeal squamous mucosa. Furthermore, we have identified several genes whose expression is altered in HPV-negative SCCHN as compared to the normal oral mucosa. Results of our study could contribute to the understanding of pathogenic mechanisms involved in the development of HPV-positive and HPV-negative oropharyngeal cancers and consequently in the identification of potential biomarkers associated with these two subtypes of squamous cell carcinomas.

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1. Introduction

There are approximately 500,000 new cases of squamous cell carcinoma of the head and neck (SCCHN) worldwide and 45,000 cases in the United States per year.[1–3] SCCHN is usually associated with such risk factors as heavy consumption of alcohol and/or tobacco. The survival in SCCHN patients is still poor and has not improved recently despite the advances in detection and therapies. It is well established that human papillomaviruses (HPVs) are involved in the pathogenesis of cervical cancer.[4–7] Recently, molecular epidemiologic studies have shown a strong correlation between oncogenic HPV infections and a subset of oropharyngeal cancers.[8–12] It is not currently understood whether these HPV infections are an independent aetiologic factor or a co-factor in the development of such tumours. Interestingly, a relative risk reduction from death of approximately 50% has been observed in HPV-associated tumours compared to those without detectable HPV DNA.[11]

HPVs are small double-stranded DNA viruses of approximately 7900 base pairs (bp). At present, more than 150 different types of HPV have been identified, and the high risk HPV types 16 and 18 are associated with a majority of cases of cervical cancer.[6,7] Replication of HPVs requires the viral E1 and E2 proteins as well as host replication factors.[13,14] The E2 protein also downregulates the transcription of the viral E6 and E7 oncogenes, which are transcribed from the p97 promoter.[15–17] The E6 and E7 oncoproteins of high-risk HPVs are involved in cellular transformation.[15–19] Most benign and low-grade cervical lesions contain HPV DNA in an extrachromosomal state.[20] However, in most cases of cervical carcinomas the HPV DNA is usually found integrated into the host chromosomes, frequently disrupting the E1 and E2 genes.[6,7,20,21] This results in increased expression of the viral E6 and E7 oncogenes.[22] The E6 protein promotes ubiquitination and consequently proteasomal degradation of the cellular tumour suppressor proteins p53 and PDZ domain-containing disc large protein (DLG).[4,7,23–25] E6 is also known to interact with a number of other cellular proteins and activates telomerase.[6,7] The E7 protein binds to and inactivates the function of pRB and related p107 and p130 proteins.[4,7] E7 also interacts with additional cellular proteins such as TBP, histone H1 kinase and cyclin E.[14–16,18,19] The E6 and E7 proteins are also known to alter cellular gene expression but the precise molecular mechanisms involved are not well understood.[16,18,19] In addition, E6/E7 expression promotes chromosomal instability, foreign DNA integration and other mutagenic events in the cell.[4,6,15,26] Although several studies have suggested a possible role for HPV infection in a subset of SCCHN, very little is known about the molecular events involved in carcinogenesis. Although the distribution of episomal and integrated HPV forms in precancerous and cancerous lesions of the head and neck has not been determined, limited evidence suggests a similar physical state as observed in cervical carcinoma. Molecular studies of HPV-associated SCCHN are necessary for a better understanding of the physical state and potential role of this virus in carcinogenesis, and for the development of new, more targeted therapeutic strategies.

Recently, DNA microarrays have been successfully used to identify global patterns of gene expression in different human neoplasias, including head and neck cancers.[27–30] In the case of breast cancer studies, a set of 70 genes correctly predicted the nature and progression of the disease as well as the outcome.[29,30] These studies support the idea that changes in the molecular profiles of gene expression are early events during carcinogenesis and such global expression profiles can be used effectively to predict the course of the disease. Recently, investigators have used microarrays to analyse gene expression changes in SCCHN tissues and cell lines, but little is known about the gene expression changes in HPV-associated SCCHN.[31–35] The identification of molecular portrait of gene expression profiles in HPV-positive and HPV-negative SCCHN, including their differences, could result in a better understanding of critical events during carcinogenesis.

This study was undertaken to identify changes in cellular gene expression profiles in HPV-positive and HPV-negative SCCHN as compared to normal tissues as well as to each other. Our microarray analysis showed considerable differences in the gene expression profiles that were specifically associated with these two types of cancers. Several of the differentially expressed genes were found to be involved in cell cycle regulation (*CDKN2A*), nuclear structure and meiosis (*SYCP2*), DNA replication and repair (*RFC5*), transcription regulation (*ZNF238*), cell differentiation (*KLK8*) and epidermis development (*CRABP2*). Unsupervised clustering analysis also showed that genes located in specific chromosomal regions such as 1p31-p36 and 12q21-q24 were specifically overexpressed in HPV-positive oropharyngeal squamous carcinomas.

2. Materials and methods

2.1. Tissue samples and cell lines

A total of 11 samples were analysed in this study: Three HPV-positive (SK20, SK30 and SK31), four HPV-negative (SK32, SK33, SK34 and SK35) and four normal oral mucosa (SK16, SK17, SK36 and SK37). Tumours and normal mucosal specimens were snap-frozen and stored at −80 °C until further use. Collection of tissues was performed under an IRB-approved Tissue Banking protocol, and written informed consent was obtained from each patient prior to sample collection. The SCCHN samples were from the oropharynx (tonsil and base of tongue), while the normal oral mucosa specimens were obtained from patients undergoing removal of the oropharyngeal tissues: tonsils, soft palate and uvula for sleep apnea. None of the patients received prior chemotherapy or radiotherapy. Cervical carcinoma cell lines were obtained from the American Type Culture Collection (ATCC) and maintained in Dulbecco's modified Eagle's medium (C-33A) or RPMI 1640 (CaSki) supplemented with 10% foetal bovine serum (FBS) at 37 °C in the presence of 5% CO_2.

2.2. Isolation of RNA and RT-PCR analysis

Total RNA from all frozen tumour samples and oropharyngeal mucosal tissues was isolated using the RNeasy Mini Kit (Qiagen Inc.) according to the manufacturer's protocol. The mucosa was carefully dissected from any subjacent muscle or lymphoid tissue before RNA extraction, so that only normal squamous epithelium was studied. The RNA pellet was dried