Differential Gene Expression Patterns In HPV-Positive And HPV-Negative Oropharyngeal Carcinomas

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ABSTRACT

Head neck cancer (HNC) is the fifth most prevalent malignancy worldwide with 90% of them categorized as squamous cell carcinomas (SCC). Carcinomas of the oropharynx, pharynx and the oral cavity, comprise a subset of HNSCC and are referred to as oropharyngeal squamous cell carcinomas (OPSCC). Up to 60% of OPSCC and 25% HNSCC are positive for high-risk human papillomavirus (HR-HPV), primarily HPV16. HPV positive and HPV negative OPSCC’s are molecularly and biologically distinct with differences in risk factors, age of presentation and clinical behavior. The precise molecular signatures of each have been well studied with respect to gene expression, genetic-epigenetic modifications and mutational analysis. However, recent studies have identified HPV as a racially linked marker for OPSCC, where African American (AA) patients present with more HPV-negative and aggressive tumors as compared to European American (EA) patients. Thus, we aimed to study the HPV status and differential gene expression (DEG) profile of OPSCC in AA and EA patients from South Carolina, using Agilent 8x60k arrays and a dual marker system for HPV status. We characterized all the tumors into HPV-active (DNA+/E7RNA+), HPV-inactive (DNA+/E7RNA-) and HPV-negative (DNA-/RNA-) based on INNO-LiPA and RT-qPCR assays, respectively. Overall, 59% of tumor samples tested positive for HPV DNA, while only 48% of those harbored an active HPV infection and significant differences
were observed when compared by race. We observed a higher prevalence of HPV in EA patients, both by DNA (69.4%) as well as by E7 RNA (39.4%), when compared to that of AA patients (40% and 10% respectively). Microarray analysis over a set of 36 oropharyngeal tumors and 4 normals revealed that HPV-inactive tumors have gene expression profiles distinct from those of both HPV-active and HPV-negative SCC, suggesting that HPV-inactive tumors may constitute a group of their own. The expression of a selected panel of genes was confirmed by RT-qPCR which was in concordance with our microarray data. Our RT-qPCR assays confirmed to the latest MIQE guidelines and data were normalized using three reference genes through Biogazelle qbase+ software and the samples were scaled to the control group (normal samples). Normalized relative quantities for each gene of interest were then calculated using qbase+. Selection of most stably expressed candidate reference genes was done through a comprehensive study of 8 well known housekeeping/reference genes through Normfinder, BestKeeper and GeNorm. Our Microarray studies and RT-qPCR assays categorized HPV-inactive tumors as a distinct entity in comparison to the HPV negative and active tumors by race. Also, HPV-inactive tumors clustered closer to the HPV-negative tumors with one exception, suggesting the loss of function of virus in former. Overall, our results confirmed that AA patients more often present with HPV-negative tumors in comparison to EA population, and indicated that HPV-negative tumors exhibit gene expression profiles indicative of activation of epithelial mesenchymal transition, while HPV active tumors are characterized by alterations of cell cycle and growth control.