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METHODS OF DETECTING HPV IN OROPHARYNGEAL CARCINOMA

INTRODUCTION

It is well known that there is large variation in the reported prevalence of human papilloma virus (HPV) in oropharyngeal squamous cell carcinoma. Part of this large variation arises from the lack of a standard assay for HPV detection. Current methods include type-specific, polymerase chain reaction (PCR) techniques, real-time PCR assays to quantitatively assess HPV viral load, and immunohistochemical detection of surrogate biomarkers such as p16 protein. In addition to various detection methods, there is also variation in the tissue type used for analysis. Specifically, both formalin-fixed paraffin-embedded (FFPE) tissue and fresh frozen tissue have been used for HPV analysis, with studies examining how these tissue differences can affect HPV detection.

OBJECTIVE

1. Understand methods of HPV detection in Head and Neck Squamous Cell Carcinoma
2. Be able to compare three methods HPV detection in oropharyngeal carcinoma: HPV DNA detection using polymerase chain reaction (PCR) in fresh frozen and formalin-fixed paraffin-embedded (FFPE) tissue samples and p16 immunostaining.

MATERIALS/METHODS

DNA was extracted from oropharyngeal fresh frozen tumor samples of 32 patients. This was analyzed for presence of HPV DNA by PCR using both the L1 universal primers MY09 and MY11 and a specific primer set to the HPV 16 E6 region. The fresh frozen samples that yielded positive results with any of these two primer sets were classified as the test group, and DNA from the corresponding paraffin-embedded samples was also tested with the same universal and HPV 16 E6 specific primers. The HPV DNA positive samples then underwent immunohistochemical analysis of p16INK4a.

RESULTS

Twenty-six of the 32 fresh frozen samples, 81%, were HPV positive using the L1 universal primers. The same 26 samples were HPV positive using the HPV 16 E6 specific primers. Of these HPV DNA positive patients, 88% of the DNA from the FFPE samples were HPV positive using the L1 universal primers and 96% were HPV positive using the HPV 16 E6 specific primers. Of the HPV DNA positive samples, 77% were p16 positive. Of the p16 positive samples, surrounding normal tissue was negative in all cases.

CONCLUSION

When using HPV 16 specific primers, FFPE and fresh frozen tissue are comparable in detecting HPV positivity. In contrast, when using L1 universal primers, FFPE is less reliable. Our results correlate with the concept of latent versus active HPV infection. Although tissue can be HPV DNA positive, the virus may not be transcriptionally active. This theory is further supported by our p16 negative normal surrounding tissue, indicative of a viral infection that is not tumorigenic.