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ORIGINAL ARTICLE

Aptima HR-HPV testing from Diff-Quick-stained fine-needle aspiration smears of oropharyngeal squamous cell carcinoma

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Introduction Human papillomavirus (HPV)-related oropharyngeal squamous cell carcinoma (SCC) is a biologically unique form of carcinoma that is important to identify for prognosis and treatment. The objective of this study was to evaluate the performance of the Aptima HPV assay using Diff-Quick (DQ) stained smears from fine-needle aspiration (FNA) of HPV-related oropharyngeal SCC.

Materials and methods Patients with a diagnosis of head and neck SCC who also had FNA sample demonstrating metastatic disease were identified. Using a mounting media-based cell transfer technique, approximately 200 tumor cells were selected and harvested from DQ-stained aspirate smeared slides. The selected cells were tested for high risk HPV using the Aptima HPV assay, an in vitro nucleic acid amplification test for the qualitative detection of E6/E7 viral messenger RNA from high-risk types of HPV. These results were compared with the p16 immunohistochemical staining of the corresponding surgical pathology specimens.

Results Twenty-eight of 32 (87.5%) FNAs of p16-positive oropharyngeal SCC were positive for high-risk HPV by the Aptima assay and 18 of 18 (100%) FNAs of p16-negative SCC were negative for high-risk HPV by the Aptima assay.

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GenProbe kits provided free of charge by Hologic, Marlborough, Mass.
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Conclusions DQ-stained FNA smears can be used by the Aptima HPV assay to accurately detect high-risk HPVs in oropharyngeal SCCs with a sensitivity of 87.5% and a specificity of 100%. This provides an alternative to p16 immunohistochemical staining of FNA cell block material, which may not be available on all specimens.

Introduction

Human papillomavirus (HPV) is a small non-enveloped DNA virus that comprises over 100 genotypes. HPV infection is common within the general population. Whereas low-risk HPV (types 6 and 11) causes non-harmful cutaneous lesions, high-risk HPVs have been found to play a leading role in the carcinogenesis of several types of mucosal malignancies including oropharyngeal squamous cell carcinoma (OPSCC).[1] Up to 80% of OPSCCs are associated with high-risk HPV infection, particularly HPV type 16. The role of high-risk HPV in carcinogenesis is associated primarily with the oncogenic activity of the E6 and E7 viral proteins.[2] These two viral proteins cooperate to cause degradation and inactivation of tumor suppressor proteins p53 and pRb, causing unlimited tumor cell proliferation.[3]

The presence of high-risk HPV in OPSCC is a strong and independent prognostic factor for patients with OPSCC. HPV-positive OPSCCs have better overall survival, a lower recurrence rate, and better response to conventional therapy compared with patients with HPV-negative OPSCCs.[4-6] Thus, the National Comprehensive Cancer Network guidelines recommend HPV testing for all primary OPSCC and head and neck SCC of unknown origin.[7] Detecting HPV can help when patients present with metastatic squamous cell carcinomas to cervical lymph nodes with unknown primary. Positive HPV status in these cases points to the oropharynx as a likely site of primary tumor.

Currently, many methods are available to detect high-risk HPVs in tissue, but none have been chosen yet as the method of choice by national standardized guidelines for fine-needle aspiration (FNA) specimens.[8] Cytopathology along with FNA play an important role in the diagnosis of head and neck squamous cell carcinomas because of the frequent presence of enlarged head and neck lymph nodes seen at presentation. In approximately 20% cases, a neck mass is the only evidence of disease at initial evaluation, making FNA the optimal choice for the initial workup. Therefore, determining the HPV status of head and neck FNA specimens is of paramount interest because of treatment outcomes for chemoradiation and surgical planning.

The surrogate marker, oncoprotein p16, has been widely used on histology material to detect transcriptionally active high-risk HPV-driven carcinoma. Immunohistochemical stains can be easily performed on cell blocks when they are available. Studies have shown, however, that the interpretation of p16 immunohistochemistry (IHC) involves determining the percentage of positive cells, which can be challenging when only a limited number of tumor cells are present on the cell block.[9-11] Alternative molecular testing for nucleic acid amplification by either polymerase chain reaction or transcription-mediated amplification (TMA) and signal amplification could improve sensitivity and specificity. In addition, the binary results (positive or negative) are simple to interpret and leaves little room for ambiguity.

Several molecular HPV tests, including the Aptima HPV assay, have been approved for the detection of high-risk HPV in cervical cancer screening. The high-risk HPV types that cause cervical carcinoma also cause OPSCC. The objective of this study is to determine if the Aptima HPV assay can be used to detect high risk HPV from Diff-Quick (DQ)-stained FNA smears of metastatic OPSCC. DQ-stained FNA smears can be rapidly made and are often used for immediate adequacy assessment.

Methods

Case selection

Archived cases of OPSCC and non-oropharyngeal SCC from 2009 to 2013 were identified from our institutional database. To be included in the study: each patient had to have both a surgical biopsy/resection and FNA of the nodal metastasis, and the DQ smears contained a minimum of 200 tumor cells. A total of 50 cases were obtained and tested for high-risk HPV by the Aptima assay.

Immunohistochemistry

IHC for p16 was performed on all resection and biopsy specimens either as part of patient care or research studies. Briefly, 4-μm-thick formalin-fixed, paraffin-embedded tissue sections were subject to primary monoclonal anti-p16 antibody (Roche MTM Laboratories), followed by incubation with secondary antibody and visualization with standard reagents. All the stains were automated using a Ventana Benchmark immunostainer. Tumors were classified as positive for p16 when $\geq$70% of tumor cells showed strong and diffuse nuclear and cytoplasmic staining.

Cell transfer from DQ-stained smears

All aspirate smears were made on uncharged slides. Selected DQ-stained FNA smears of SCCs were scanned