

Emerging Markers from Molecular Studies: Application into Routine Salivary Gland Fine Needle Aspiration Biopsy Practice

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Introduction

Salivary gland neoplasm (SGN) is one of the largest groups of neoplasms, with over 3 dozen benign and malignant tumors in the 2005 WHO classification and with additional entities recognized during the last decade. Current success of fine needle aspiration biopsy (FNAB) in SGN diagnosis is largely because of its high sensitivity and specificity in: 1) distinguishing neoplastic from non-neoplastic salivary gland lesions and 2) distinguishing benign and low grade salivary gland neoplasms from high grade malignancies. However, precise subtyping of SGNs remains a diagnostic problem for some FNAB cases. These challenging cases include: 1) low grade malignancies with minimal malignant cytological features; 2) accurate subtyping of high grade malignancies, including metastatic malignancies; 3) benign SGNs that can only be differentiated from their malignant counterparts by growth pattern evaluation (e.g. basal cell adenoma vs. basal cell adenocarcinoma).

Emerging molecular markers including specific chromosomal translocations, and evolving technologies such as fluorescence in situ hybridization (FISH), reverse transcription polymerase chain reaction (RT-PCR) and next generation sequencing (NGS) provide real hope to improve the diagnostic accuracy of salivary gland FNAB. Compared to classic immunohistochemical markers for various SGNs, including c-Kit for adenoid cystic carcinoma (ACC) and p63 for mucoepidermoid carcinoma (MEC), genetic changes can be highly specific for a particular subtype of SGNs. Genetic changes in various SGNs has been excellently summarized in recent reviews [1,2]. The most common changes of great diagnostic value include gene rearrangements of PLAG1 (pleomorphic adenoma gene 1) and HMGA2 (high mobility group AT hook 2) for pleomorphic adenoma (PA), MAML2 (mastermindlike 2) for MEC, Myb (v-myb avian myeloblastosis viral oncogene homolog) for ACC, ETV6 (ets variant 6) for mammary analogue secretory carcinoma (MASC) and PRKD1 (serine/threonine-protein kinase D1) for cribriform adenocarcinoma. Also of high diagnostic utility is gene amplification of ERBB2 (receptor tyrosine-protein kinase erbB-2 precursor) for salivary duct carcinoma, PRKD1 mutation for polymorphous low-grade adenocarcinoma and CTNNA1 (catenin) mutation for non-membranous type basal cell adenoma and adenocarcinoma.

A series of recent studies have made initial efforts to translate the newly identified markers into improved diagnostic accuracy of routine salivary gland FNABs. Immunohistochemical staining is an integral part of modern pathological diagnosis worldwide. Commercially available antibodies to various fusion proteins and products of gene

rearrangements are becoming increasingly available. Pusztaszeri et al. [3] and Foo et al. [4] both showed antibodies to Myb, PLAG1 and HMGA2 are useful in distinguishing ACCs from PAs. Actually Foo et al [4] reported that when both Myb and c-Kit were positive, and both PLAG1 and HMGA2 were negative, there was a specificity and positive predictive value of 1.0 for ACC. Interestingly, a subset of ACCs without detectable Myb related gene rearrangement also overexpressed Myb mRNA and protein. Certain microRNAs (miRs) including miR-15a, miR-16 and miR-150 are suggested to participate in the upregulation of Myb. Similarly, overexpression of PLAG1 protein has been identified in a subset of PAs without detectable PLAG1 gene rearrangement. Specific antibodies to MECT1 (mucoepidermoid carcinoma translocated-1)-MAML2 fusion protein specific for MECs are also now available; but systematic evaluation of their diagnostic utility has not been reported.

FISH is a classic method for cytogenetic analysis, actually the original identification of Myb-related t-chromosomal translocation in ACC was achieved in surgically excised neoplasms using break-apart FISH probes [5]. Salivary gland FNAB cellular smears provide excellent starting material for FISH analysis. Using FISH and salivary gland FNAB smears, Hudson and Collins [6] demonstrated 50% (5/10) of ACC cases contained Myb gene rearrangement or trisomy; while no such rearrangement or trisomy was identified in 13 cases of PAs. Griffith et al. [7] detected ETV6 gene rearrangements in the cell blocks of all 3 tested MASC cases. While FISH analysis is highly specific, this technology is not readily available in every pathology laboratory.

PCR has been applied to the cytological diagnosis of salivary gland low grade lymphoma as early as 1998 [8]. The impressive power of RT-PCR to amplify gene copy number is very attractive for many cytopathologists, especially because the FNAB specimens frequently contain limited tumor cells. However complex genetic changes, including probably unknown fusion gene partners might hinder current application of this technique into routine practice. A recent RT-PCR based study using primers of all known PLAG1 and HMGA2 fusion gene partners identified chromosomal translocation in only 12/45 PA cases [9]. On the contrary, all 45 PA cases are immunohistochemically positive for PLAG1 protein. Amplification of genes downstream of the gene rearrangement products might be an alternative approach to apply RT-PCR into routine FNAB diagnosis of SGNs.

NGS can be successfully conducted by using as little as 5-10 nanograms of DNA, well within the range of DNA obtained by FNAB. In combination with anchored multiplex PCR, NGS can detect gene rearrangement even without prior knowledge of the fusion partners, insertion mutation, gene deletion, or copy number changes. Pilot studies using targeted NGS has already identified novel genetic changes fundamental to salivary gland tumor classification and

treatment [10]. With reduced cost of NGS, its foreseeable application in routine FNAB diagnosis will significantly improve diagnostic accuracy.

FNAB is still the initial diagnostic method of choice for many SGNs. When accurate diagnosis cannot be achieved by cytomorphology and classic immunohistochemical markers, molecular markers developed from recent genetic studies are of critical value. FISH and NGS are powerful techniques to detect chromosomal translocations, gene deletion and amplification; immunohistochemistry and PCR are proven approaches to detect oncoproteins/oncogenes resulting from chromosomal translocation. Our routine salivary gland FNAB practice will be significantly empowered by the utilization of new molecular markers, thus improving accuracy in the heterogeneous territory of SGN diagnosis.

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