Applications of molecular testing in surgical pathology of the head and neck

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Molecular testing in routine surgical pathology is becoming an important component of the workup of many different types of tumors. In fact, in some organ systems, guidelines now suggest that the standard of care is to obtain specific molecular panels for tumor classification and/or therapeutic planning. In the head and neck, clinically applicable molecular tests are not as abundant as in other organ systems. Most current head and neck biomarkers are utilized for diagnosis rather than as companion diagnostic tests to predict therapeutic response. As the number of potential molecular biomarker assays increases and cost pressures escalate, the pathologist must be able to navigate the molecular testing pathways. This review explores scenarios in which molecular testing might be beneficial and cost-effective in head and neck pathology.
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Molecular pathology has become an essential tool in surgical pathology in many different tumor types, and diagnoses molecular assays have become the standard of care for diagnosis and management of certain malignancies.[1,2] At the same time, we are experiencing an exponential growth in the number of tumor assays available, we are also experiencing financial and cost pressures nationally.[3,4] Across medicine, efforts are being made to reduce cost, while improving quality and efficiency and patient satisfaction. These goals were codified in a 2008 Institute of Medicine report that described the so-called triple aim:[5–7]

- Improving the patient experience of care, including quality and satisfaction
- Improving the health of populations
- Reducing the per capita cost of health care

In recent years, national payment reform efforts have been escalating to address the cost side of the triple aim, with programs including expand bundled payment models, capitation, and accountable care.[3,8–12] In these payment models, every additional intervention can be seen as representing added cost (not revenue). In pathology, this means that every added test ordered represents reduced overall revenue from a fixed payment, which is unlike the traditional fee-for-service model, but similar to current models for diagnosis-related group payments.[13,14] Utilization management efforts need to be directed at maximizing quality and minimizing cost through reduction of unnecessary or non-contributory testing. Although the 'gatekeeper' role for influencing test ordering practices has not been seen as enviable in the past, new payment models may encourage pathologists to take on utilization management to help provide value in managing limited resources for organizations and groups.[15–17]

Because of external and internal pressures, the pathologist today needs to not only be familiar and comfortable with the wide array of molecular tests available, but also needs to be able to critically examine the rational for molecular testing and understand the value that can be provided (or not) in specific scenarios. In head and neck pathology, there are molecularly based markers for almost every type of tumor and disease. Many studies describe markers with putative prognostic value.[18,19] There are also diagnostically useful markers. The most powerful markers, which are limited in head and neck pathology today, are those that directly affect therapeutic decision making. This review will focus on selecting cost-effective and clinically useful molecular assays, illustrated with several head and neck pathology examples.

Molecular testing for diagnosis

Early approaches in molecular diagnostics were focused on mutations that were specifically

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associated with a disease or condition.[20–22] These diagnostic assays have been important drivers in pathologists' ability to develop refined classification systems and provide more exact diagnoses. Early testing was hampered by the difficulties in obtaining enough fresh tissue for available approaches.[22] With the widespread implementation of polymerase chain reaction and fluorescent *in situ* hybridization and now even more advanced technologies, routine testing has become much simpler to perform on paraffin-embedded material.

Many common diagnostic tests today involve tumor-associated oncogene mutations, such as translocations and point mutations.[23–26] Some of the best studied translocations are those associated with hematopoietic malignancies and sarcomas.[23] In the head and neck, one can encounter these hematopoietic and soft tissue malignancies and molecular testing will be used for diagnosis, often paired with other diagnostic markers, such as flow cytometry and/or immunohistochemistry.

In recent years, translocations have been increasingly identified in epithelial-derived solid tumors. In the head and neck, several tumor-associated translocations have been defined in salivary gland tumors.[27–29] These translocations appear to be relatively specific, and most have a relatively high prevalence within their tumor category (Table 1). Interestingly, several of these translocations were first identified years ago based on classical cytogenetic studies. It was not until fluorescent *in situ* hybridization technologies allowed for the study of paraffin-embedded tissue samples that the surprisingly high percentage of cases harboring the translocations was truly appreciated.[30–33]

Molecular testing for therapeutic decision making

Another recent advance in oncologic molecular diagnostics has been the identification of clinically useful molecular assays to predict responsiveness or resistance to specific therapies. As a general category, these markers are often referred to as 'companion diagnostics.'[34–36] Companion diagnostics have received national press in the past few years because of the Food and Drug Administration's proposal to implement oversight laboratory developed tests, with a particular focus on companion diagnostics.[4,37,38]

Table 1 These are the common translocations that have been identified in salivary gland tumors

Tumor type	*Translocation*
Mucoepidermoid carcinoma	MECT1-MAML2
Adenoid cystic carcinoma	MYB-NFIB
Mammary analog secretory carcinoma	ETV6-NTRK3
Clear cell carcinoma	EWSR1-ATF1

In head and neck cancer, relatively few companion diagnostic assays are used in the routine clinical setting. For example, despite the fact that squamous cell is extremely common, there are very few molecular assays for squamous cell carcinoma that are used for therapeutic decision making. The most widely studied markers in head and neck squamous cell carcinoma (HNSCC) with prognostic implications are viral markers for Epstein–Barr virus and human papillomavirus (HPV).[39–41] These markers can be used as diagnostic tools, to help subclassify squamous cell carcinoma variants in specific anatomical subsites. It has also been recognized that viral-associated squamous cell carcinomas may have a different prognosis than tobacco associated HNSCC, but they may also respond in a different way to radiation and chemotherapy.[41–43] These observations have led to ongoing clinical trials using HPV testing to identify patients for de-escalation therapy.[44,45] Currently, this therapeutic approach is being used in the clinical trial setting, but more widespread use is likely if the trials are successful.

Another exciting avenue for molecular oncology testing has arisen from the advent of novel testing platforms using new technologies for next-generation sequencing, or massively parallel sequencing.[46–48] With the ability to sequence the entire exome (or the entire genome) in a rapid, cost-effective and highly sensitive manner, more and more mutations are being identified in human malignancies. These techniques are particularly useful to identify low prevalence mutations with potential therapeutic targets agents.[49] Recent work from the Cancer Genome Atlas project, which undertakes next-generation sequencing for specific tumor types, has given us a deeper understanding of the mutations that can be seen in HNSCC.[50–52] Although this may lead to more extensive and expanded clinical testing platforms, it may also lead to better selection of drugs, either as stand-alone therapy, or as combination therapies.[53]

Clinical applications for molecular testing in head and neck pathology

The challenge for the pathologist is not just knowing the relevant mutation profiles for different tumors, but also in truly understanding the practical value for patient care and being able to assess the benefit of any given mutation panel. Perhaps the most important question that a pathologist can ask before ordering a molecular test is: how will this test result change the management of this patient? There are many cases in which a molecular test can be done; the pathologist must know when the molecular test should be done. The scenarios when molecular testing is a cost-effective and high value addition to the diagnostic workup are broad and varied, and a

case-by-case approach is likely needed. For example, the pathologist who is facing a challenging differential diagnosis, including both tumors with different management protocols, may find a diagnostic molecular test extremely useful. But, in a differential diagnosis where the management would be the same, the molecular test might be simply added unnecessary cost. In cases where novel targeted therapies or alternative approaches are being explored, molecular testing, particularly in the setting of companion diagnostics, may be necessary. In other cases, where there are no available targeted therapies, the testing may not be important. In the ensuing section, a few illustrative example cases will be explored, and the decision making surrounding molecular testing will be discussed.

Case 1

A 54-year-old female presented with a partially cystic mass of the parotid gland. The tumor was resected and the margins were negative. The histology demonstrated a lesion with three cell types, including mucous cells, epidermoid cells, and intermediate cells. The diagnosis of low-grade mucoepidermoid carcinoma was made (Figure 1).

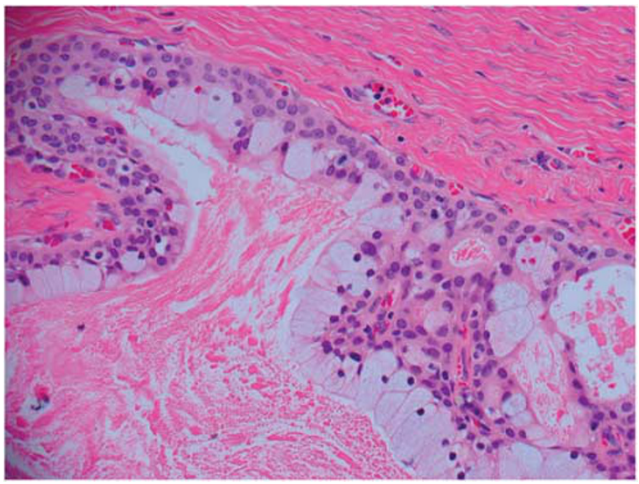

Figure 1 This image is a high power view of the lining of the cystic neoplasm in case 1. The epidermoid and mucous cells are apparent.

Case 2

A 54-year-old female presented with a solid and cystic mass in the parotid gland that was resected. The histology demonstrated a complex cystic lesion with abundant oncocytic cells, some of which were lining papillary structures. There were also areas with islands of mucous cells and epidermoid cells. The lesion was surrounded by a dense lymphoid stroma. A translocation analysis demonstrated a positive result. The diagnosis of mucoepidermoid carcinoma was made, and a comment mentioned the possibility of a Warthin-like variant morphology[54,55] (Figures 2 and 3).

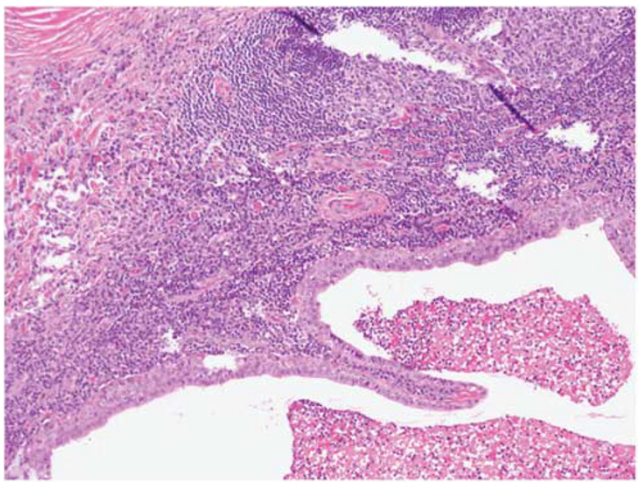

Figure 2 This area of case 2 the lesion shows an oncocytic lining with a lymphoid stroma.

Commentary. It has been well established that mucoepidermoid carcinomas can harbor a specific translocation, the *MECT1-MAML2* (*CRTC1/3-MAML2*).[29,56] The translocation is more common in low and intermediate grade tumors, but can also be found in high-grade mucoepidermoid carcinoma. Another translocation, the *EWSR1-POU5F1* has been identified in a subset of high-grade mucoepidermoid carcinomas.[57,58] These translocations are not seen in mimickers of these tumors, such as adenosquamous carcinoma.[59] The most common testing approach uses break-apart fluorescent *in situ* hybridization probes. The assay can easily be performed on fresh tissue, paraffin-embedded tissue, and even on cytological samples.

Though this assay is straightforward to perform and interpret, in most cases of routine mucoepidermoid carcinoma (such as case 1 above), there is very

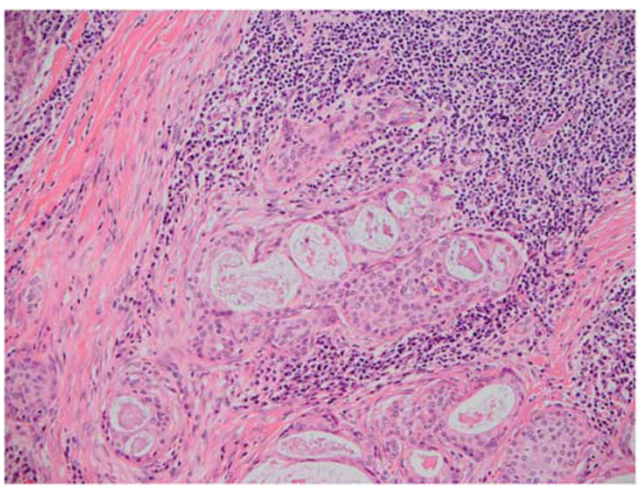

Figure 3 This area of case 2 shows a more typical area of mucoepidermoid carcinoma, with epidermoid cells, mucous cells, and intermediate cells.

little role for testing. When the diagnosis can be made on the H&E slide, identifying the translocation will not change patient management and will add cost to the workup of the tumor. With no current role for targeted therapy, particularly in the low and intermediate grade tumors, the test is of limited value in this setting.

In the second case, the diagnosis is not as straightforward and the pathologist may consider a differential diagnosis of a Warthin tumor with metaplasia and a Warthin-like mucoepidermoid carcinoma. Early studies demonstrated suggested that Warthin tumors with metaplasia could harbor the translocation,[60] although other studies did not have this finding.[61] Further investigations have suggested a more likely explanation is the presence of a Warthin-like variant of mucoepidermoid carcinoma.[54] In case 2, a molecular assay to test for the translocation, particularly with the positive result, was a useful diagnostic biomarker. In the highly specific scenario of a challenging variant morphology where the differential diagnosis included a benign entity, the presence of the translocation would be of clinical benefit.

Case 3

A 38-year-old male presented with a parotid mass lesion. A superficial parotidectomy was performed and showed a solid tumor with predominantly clear cells. An immunohistochemical workup was performed, which demonstrated that the tumor was negative for p63, pankeratin, CAM5.2, SMA, calponin, and CK5/6. The tumor was positive for CD99. An *EWSR* translocation analysis was positive and a diagnosis of Ewing's/PNET was made (Figures 4 and 5).

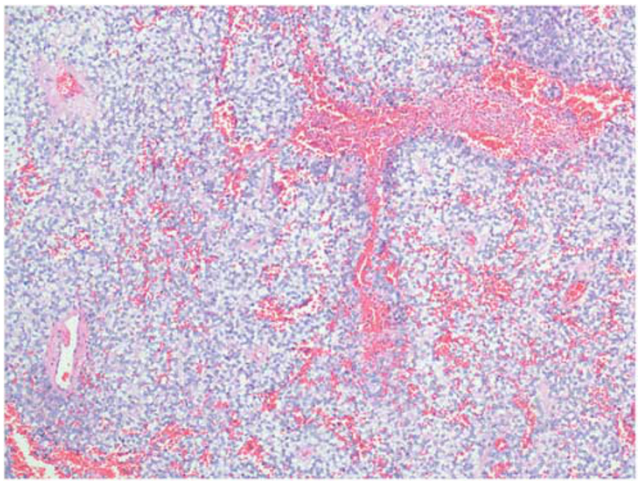

Figure 4 This is a low power view of the clear cell tumor in case 3.

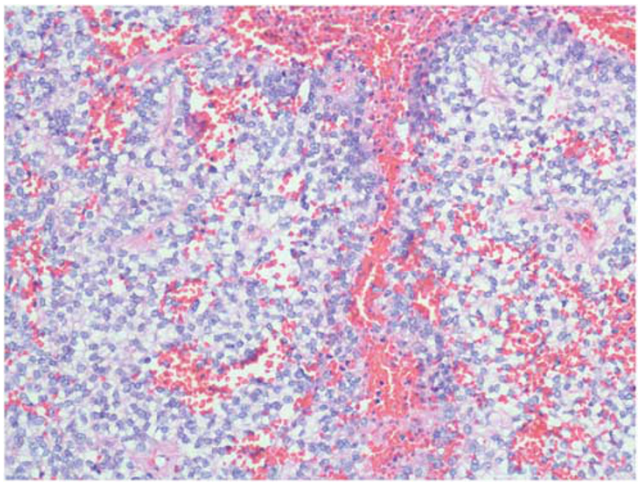

Figure 5 This is a high power view of the clear cell tumor in case 3.

Commentary. Based purely on the morphology of this tumor, the pathologist might consider a fairly broad differential diagnosis. Primary tumors of epithelial origin, such as clear cell carcinoma, clear cell mucoepidermoid carcinoma, and clear cell myoepithelial tumors would all be considered. Metastatic renal cell carcinoma can occasionally be found in the head and neck, though the parotid is an exceptionally rare site.[62] Finally, there are some mesenchymal tumors that can have a clear cell phenotype, including Ewing's/PNET.[63]

Interestingly, molecular assays can help to distinguish most of the entities in the differential diagnosis above. Clear cell carcinomas were recently found to harbor *EWSR1-ATF1* translocations.[56,64] These tumors, however, would be expected to stain with p63 and cytokeratin, unlike the case described above.[65] Clear cell mucoepidermoid carcinoma would harbor the *MECT1-MAML2* translocation (see above discussion). And, clear cell myoepithelial tumors also harbor *EWSR1* rearrangements.[66,67] Thus, coupled with the unique immunoprofile, the presence of an *EWSR1* rearrangement is useful diagnostically and will enable the correct management of the patient.[68]

Case 4

A 45-year-old male presents with a tumor of the sinonasal cavity. Histologically, the tumor has an invasive border, with both stromal and extensive perineural invasion. The tumor had both cribriform and tubular areas, and also a solid component. The tumor is biphasic, with both epithelial and myoepithelial cells on immunohistochemical stains. HPV *in situ* hybridization was negative. The diagnosis of adenoid cystic carcinoma was made (Figure 6).

Commentary. The differential diagnosis in this case is fairly limited, with the most common entity being adenoid cystic carcinoma. However, a recently

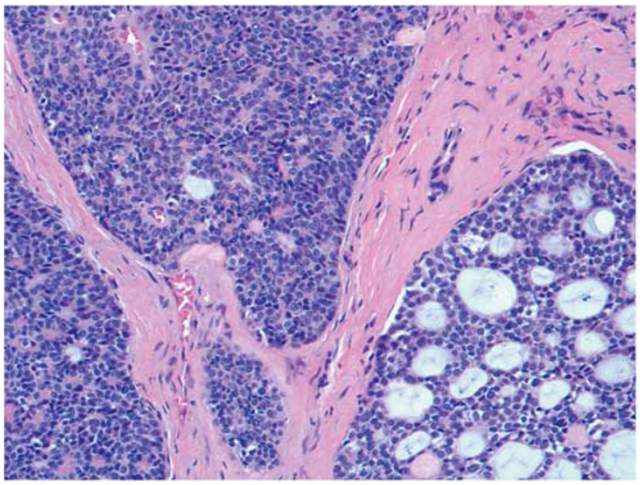

Figure 6 This shows a cribriform and solid area of the tumor in case 4.

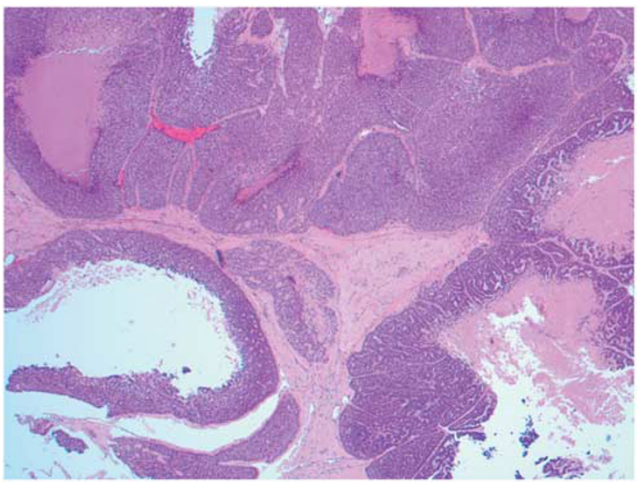

Figure 7 This is a low power view of the tonsil carcinoma in case 5.

described tumor of the sinonasal tract is also included in this differential diagnosis, HPV-associated adenoid cystic-like carcinoma of the sinuses.[69] In this setting, an HPV test was useful to rule out this unusual lesion. In the absence of HPV, a diagnosis of adenoid cystic carcinoma can be made.

It has also been recognized that adenoid cystic carcinomas harbor a unique translocation, the MYB-NFIB.[29,70,71] In this case, which has a fairly straightforward morphology and immunohistochemical staining profile, the translocation test will add very little value. But, this could change in the future, if targeted therapeutic approaches evolve. Because adenoid cystic carcinoma is a relentless malignancy that tends to be difficult to cure,[72–74] there have been some attempts to use targeted therapy in adenoid cystic carcinoma. The first of these was based on the fact that the vast majority of adenoid cystic carcinomas over-express CKIT (CD117) by immunohistochemistry. Early attempts using drugs such as imatinib and dasatinib were not highly successful.[75–78] Further investigation into the molecular biology of adenoid cystic carcinoma revealed why the therapy did not work well. Adenoid cystic carcinomas did not harbor any mutations in the *CKIT* gene.[79–81] There are currently clinical trials exploring potential novel therapies for adenoid cystic carcinoma with targeted therapies based on the presence of the *MYB-NFIB* translocation.[70,82,83] If these therapies prove to be effective in treating this tumor, there may be role in the future for identifying the translocation to triage patients for therapeutic management.

Case 5

A 58-year-old male presents with a cystic mass in the neck, which did not respond to antibiotic therapy. A fine needle aspiration biopsy was done and a diagnosis of metastatic squamous cell carcinoma was made. A p16 stain was positive. Further clinical investigation revealed a small squamous cell carcinoma in the tonsil that was found to be HPV positive. The patient was treated for with radiation and chemotherapy, but subsequently recurred in the neck and then developed new metastatic lesions in the brain. This patient's tumor was tested for a mutation panel for oncogenes with available targeted therapy approaches. The tumor harbored a *PIK3CA* mutation and the patient was entered into a clinical trial (Figure 7).

Commentary. Our understanding of the mutational landscape of HNSCC is evolving, but it is now recognized that a number of tumor-associated oncogenes can be mutated in these tumors. Although some of these mutations are rare, others have a higher prevalence. But, even for uncommon mutations, there may be clinical importance, especially in the setting of failed conventional therapies. For example, PIK3CA mutations are seen in ~5–10% of HNSCC.[84–87] Several clinical trials have investigated drugs targeting *PIK3CA* and have shown some promising results.[53,86,88,89] Most studies using targeted therapies for rare mutations are still in the early phases, but it is expected that targeted therapy will become a clinical option for treatment in HNSCC.[53,90]

Conclusion

Molecular testing is becoming more and more common in surgical pathology practice. In the head and neck, there are some current and upcoming promising assays for both diagnosis and therapeutic planning. Testing should be performed only in high-value scenarios, where the outcome of the test impacts patient management.

Disclosure/conflict of interest

The author declares no conflict of interest.

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