Molecular Diagnosis in Head and Neck: What a Surgical Pathologist Must Know

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Abstract Molecular alterations in tumors have become interesting targets both for diagnostic and for therapeutic and prognostic applications in tumor pathology. In the head and neck, there are a variety of different alterations, encompassing all the different types of genetic events associated with carcinogenesis. This paper reviews three different types of tumors that display a spectrum of genetic alterations: the translocation in Mucoepidermoid carcinoma, Epstein Barr virus association in nasopharyngeal carcinoma, and the HRPT2 tumor suppressor gene in parathyroid carcinoma. Basic histology is reviewed and the genetic alterations are discussed, along with a brief discussion of potential diagnostic implications.

Keywords Tumor markers · Mucoepidermoid carcinoma · Lymphoepithelial carcinoma · Nasopharyngeal carcinoma · Parathyroid carcinoma

Mucoepidermoid Carcinoma: Illustration of a Translocation

Mucoepidermoid carcinoma (MEC) is the most common salivary gland malignancy; it represents between 2 and 16% of all salivary gland tumors and up to 1/3 of malignant salivary gland tumors [1]. Histologically, MEC carcinoma has three cellular components: mucus cells, epidermoid cells, and intermediate cells. Grading is extremely important in MEC, as it correlates with prognosis in almost every

study that has been done. The grading systems have varied over time. The first grading system suggested that only the amount of cyst content had to be examined. Tumors with more than 90% cyst content were considered to be low grade and those with less than 90% were considered to be high grade [2, 3]. Later grading systems converted this to a three-tiered system, with low, intermediate, and high-grade tumors [4]. The three-tiered grading system has been validated by several additional series that consistently show correlation between tumor grade and prognosis [5–8].

Mucoepidermoid carcinoma is treated primarily with surgery. Grade 1 tumors do not require additional therapy or lymph node dissection. High-grade lesions, especially grade 3 tumors, may be treated with cervical lymph node dissection, and grade 2 tumors do not have standardized therapy. Adjuvant radiation therapy may be used for high-grade tumors and for patients who have histologically positive surgical margins.

A very interesting finding has been the discovery of a translocation in MEC. The t(11;19)(q21-22;p13) translocation between WAMTP1-MAML2 disrupts the Notch signaling pathway [9–12]. Recent evidence suggests that this translocation may have prognostic relevance, since it is only seen in low and intermediate grade MEC and not in high-grade tumors [10] (Table 1).

Although many MECs are straightforward, there are tumors in which a diagnostic marker would be useful for diagnosis. Because of the strong association between MEC and the presence of this specific translocation, the potential exists for a diagnostic application. Furthermore, because the translocation is seen in the low and intermediate grade tumors, there is also potential for prognostic significance. Reverse transcription and polymerase chain reaction (RT-PCR) can be used to detect the translocations, or alternatively fluorescent in situ hybridization (FISH) can be used.

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Table 1 Presence of translocation in MEC in comparison to grade of tumor

Grade	Positive	Negative	Rate (%)
Grade I	41	21	66
Grade II	8	11	73
Grade III	0	28	0

Data collated from [10–12]

Currently, there are no commercially available FISH probes, but this will clearly be the most straightforward test for the translocation.

Nasopharyngeal Carcinoma: Illustration of Viral Etiology

Lymphoepithelial carcinoma (LEC) is the descriptive name for the tumor that has also been referred to as “Nasopharyngeal undifferentiated carcinoma.” It has been variously named in the past as Regaud and Schmincke’s tumor, undifferentiated nasopharyngeal carcinoma (in the nasopharynx), and lymphoepithelioma. Nasopharyngeal LEC or those that involve Waldeyer’s ring (base of tongue, palatine tonsils, and adenoids) are more common in Southeast Asia and North Africa, where the incidence may be as high as 30–80/100,000 [13]. In the USA and Europe the incidence is well below 1/100,000. In Asia, there is a bimodal age distribution, with the largest peak in the sixth decade and the smaller peak between 10 and 25 years of age [14]. The most common presentation is a mass in the neck, with possible cranial nerve involvement and resulting neurologic symptoms.

In non-nasopharyngeal sites, LEC is quite rare. In several large series of all laryngeal carcinomas, LEC represented less than 0.2% of the cases studied. In our experience, the most common non-nasopharyngeal site is the larynx, and particularly the pyriform sinus.

The histologic features of LEC are unique. The cells in these tumors are large, and pleomorphic. The oval to round nuclei are vesicular and may contain large, distinct nucleoli. The cells grow in syncytial sheets that have prominent infiltrating lymphocytes. The lymphocytes in these lesions are polyclonal and therefore are considered to be reactive in nature. Two types of lesions have been described: pure LEC and mixed LEC with a component of conventional SCC. No differences in prognosis have been described, though this tumor is rare and large series have not been reported.

Lymphoepithelial carcinoma in the nasopharynx is highly associated with Epstein–Barr Virus (EBV), particularly in endemic areas, such as Southeast Asia and North Africa [15]. In non-endemic areas, EBV is present in

approximately 1/3 of cases [16, 17], but this percentage can be higher in areas of high immigration. Tumors in non-endemic areas are more often smoking related. The age distribution in western countries is unimodal (sixth decade predominance), which supports the fact that it parallels conventional squamous cell carcinoma.

Genetic predisposition to nasopharyngeal LEC has been suggested by correlation between development of tumors and certain HLA profiles [18, 19]. The at-risk HLA types appear to be different from region-to-region. Diet has also been implicated as having a role in the development of nasopharyngeal LEC. Salted fish and preserved foods containing nitrosamines and herbal teas are a few of the suspected agents [20–22].

Testing for EBV in tissue sections is usually done using in situ hybridization (ISH). There are several commercially available probes that produce very high-quality results.

Parathyroid Carcinoma: Illustration of a Tumor Suppressor Gene

The majority of patients with primary hyperparathyroidism present with incidental hypercalcemia, found at routine health screenings. A sestamibi scan can help the surgeon to pre-operatively locate the abnormal gland, but these may be non-specific and thus exploration during surgery may still be needed.

Parathyroid adenoma will have typical histologic findings. The cellular composition may show predominantly a single cell type, or can show mixed features [23]. Other morphologic features of parathyroid adenoma may include a rim of normal somewhat suppressed parathyroid tissue around the outside of the gland. This can be used as a diagnostic clue for the etiology of the pathologic process. Mitoses are usually rare to absent in parathyroid adenomas [24, 25].

The differential diagnosis between benign adenoma and carcinoma cannot be reliably made just based on the histologic assessment alone (Table 2). The surgeon’s intraoperative opinion about adherence to surrounding

Table 2 Clinical and histologic features of parathyroid carcinoma

Clinical features	Histologic features
High calcium level (>14 mg/dl)	Trabecular growth
Parathyroid hormone level >5× normal	Broad intersecting fibrous bands
Palpable mass lesion	Increased mitoses
Bone symptoms	Stromal invasion
Operative findings of invasive growth (sticky, fibrotic, vascular gland)	Angiolymphatic or perineural invasion

structures is an important criterion in distinguishing benign from malignant parathyroid neoplasms. The surgeon who encounters a parathyroid carcinoma will describe the gland as “sticky,” “fibrotic,” “hypervascular,” or “adherent to local structures” [26, 27]. These descriptions should immediately alert the pathologist to the possibility of parathyroid carcinoma. The histologic features of malignancy may not all be seen in a given case [25, 28]. There are some histopathologic features which are associated with malignancy, though they are certainly not pathognomonic for carcinoma. Worrisome features include the presence of increased or atypical mitoses, broad bands of fibrosis, trabecular growth pattern, invasion of adjacent tissue, and perineural or angiolymphatic invasion [29]. These features usually correlate with malignancy, though these histologic features are not always present in every case of parathyroid carcinoma [25, 30, 31]. Parathyroid carcinomas tend to be locally invasive and invasion is most commonly seen into the thyroid gland, strap muscles, recurrent laryngeal nerve, esophagus, or trachea [25, 32].

There have been some studies of the molecular mutational findings in parathyroid neoplasia. One feature that is consistently noted is that parathyroid adenomas and carcinomas have a high rate of loss of the short arm of chromosome one (1p) [33–37]. This is not a feature that is generally seen in parathyroid hyperplasia. Other genes have also been implicated in parathyroid adenomas and carcinomas, including *Retinoblastoma (RB, 13q14.3)*, the *MEN* gene (11q13), and the *BRCA2* gene (13q12.3) [38–40].

Studies of a very interesting syndrome (hyperparathyroidism—jaw tumor syndrome) have provided insight into the pathogenesis of parathyroid neoplasia. The syndrome includes parathyroid cysts, parathyroid carcinomas, and fibro-osseous lesions of the jaw. Although it is reportedly rare, it is probably under-recognized. The syndrome is associated with mutations in a tumor suppressor gene mapping to 1q25-31 and designated as *HPRT2*. This gene harbors germline mutations in hereditary cases of this syndrome [34, 41]. Loss of heterozygosity and somatic point mutations have been detected in sporadic parathyroid carcinomas, as well [34, 42].

Interestingly, the protein product for the *HPRT2* gene, parafibromin, has been recently identified as a potential marker for parathyroid carcinoma. Expression of parafibromin is lost in between 70 and 90% of both syndromic and sporadic parathyroid carcinomas [43–45]. Parafibromin is almost always preserved in histologically and clinically benign parathyroid adenomas [46]. There are commercially available antibodies for parafibromin, but most laboratories are currently not offering testing at the DNA level for tissue samples.

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