Introduction
Molecular cytogenetics involves, among other things, the study of chromosomal abnormalities at the level of DNA. Chromosome translocations are genetic abnormalities caused by the transfer of a chromosomal segment to a new position, especially on a different chromosome. Translocations can result in the overexpression of one gene by placing it next to a routinely expressed gene. A common scenario in tumorigenesis is the fusion of a proto-oncogene with a promoter gene, resulting in overexpression of a functional fusion protein that leads to cell immortalization. These are acquired genetic events, as opposed to congenital germline or syndrome-producing mutations. Tumor-specific chromosomal translocations have important diagnostic, prognostic and therapeutic implications and are now considered to be the defining features of a wide variety of hematogenous and soft tissue tumors.

More recently, there has been significant progress in understanding the cytogenetics of salivary gland tumors. Mucoepidermoid carcinoma and pleomorphic adenoma, the two most common salivary gland tumors1; along with adenoid cystic carcinoma, mammary analogue secretory carcinoma and hyalinizing clear cell adenocarcinoma all harbor specific and characteristic translocations (Table 1).

Diagnostic Methods
A variety of molecular laboratory techniques can be employed to identify chromosomal translocations. Polymerase chain reaction involves amplification of the genetic sequence of interest using specific DNA primers.2 In situ hybridization (ISH/FISH) utilizes paired fluorescent-tagged DNA primers that bind to both contributors of a fused gene. Both techniques can be applied to paraffin-embedded biopsies but ISH can be used directly on a microscopic slide. It is rapidly becoming the standard technique for identifying translocations.2

Adenoid Cystic Carcinoma
Adenoid cystic carcinoma (ACC) is a malignant salivary gland tumor with a propensity for perineural invasion, a female predilection and a peak incidence in the sixth decade.1 It is not uncommon and most often involves the palate or parotid gland.1 The prognosis is typically good for the first five years, however, persistent growth and late metastases result in a poor 10-20 year survival rate.5

Recently, a t(6;9)(q22-23;p23-24) translocation was identified in a subset of adenoid cystic carcinomas.6 This translocation corresponds to a gene fusion event between NFIB, a transcription factor3 and MYB, a potent oncogene identified in malignancies of pancreas, breast, and hematopoietic tissues (leukemia).4 The result is dramatically increased expression of MYB.4 High MYB expression has been correlated with poorer survival in ACC patients.5 At this time, assured surgical excision, close monitoring and radiation therapy as required are the recommended therapy for isolated tumors1, however, targeting downstream MYB-NFIB products and proposed DNA vaccines against the MYB gene segment may provide future therapeutic approaches.4

Hyalinizing Clear Cell Adenocarcinoma
A relatively rare tumor, hyalinizing clear cell adenocarcinoma (HCCA) is a low grade malignancy which primarily occurs in the minor salivary glands of older males and females.1 Histologically, it is composed of cords and nests of clear epithelial cells embedded in a hyalinized stroma.6

Current evidence indicates that a t(12;22)(q13;q12) gene translocation differentiates HCCA from other clear cell tumors such as mucoepidermoid carcinoma, myoepithelial carcinoma and epithelial-myoepithelial carcinoma.7 Interestingly, a relationship between HCCA and clear cell odontogenic carcinoma has recently been described. The t(12;22) translocation was identified in 82% of HCCA cases and 83% of clear cell odontogenic carcinoma cases, suggesting they may represent a central variant of HCCA.7,8

The t(12;22) translocation results in an EWSR1-ATF1 fusion that is also present in clear cell sarcomas and angiomatoid fibrous histiocytomas.5 EWSR1 encodes a multifunctional protein that is involved in various cellular processes, including gene expression, cell signaling and RNA processing. It is notable for its tumorigenic role in Ewing sarcoma and related tumors.9

Mammary Analogue Secretory Carcinoma
Mammary analogue secretory carcinoma (MASC) of salivary glands was first described in 2010 by Skalová and colleagues after identifying 16 salivary gland tumors that had previously been categorized as acinic cell adenocarcinomas or cystadenocarcinomas.10 Microscopically, the tumors resemble secretory carcinoma of the breast and demonstrate a lobulated growth pattern with microcystic and glandular spaces filled by eosinophilic secretory product.10 They occur primarily in the parotid gland with a slight male predilection and a mean age of 46 years at time of presentation.10,11

MASC harbors a t(12;15)(p13;q25) translocation, which results in the ETV6-NTRK3 fusion oncogene,10 encoding a chimeric tyrosine kinase, that has also been identified in secretory breast carcinomas, congenital fibrosarcoma, leukemias and other malignancies.10-12 Although MASC typically demonstrates clinical features and outcomes similar to the low-grade acinic cell adenocarcinoma, targeting the chimeric tyrosine kinase protein may be a future therapeutic option.10,12

Cytogenetics of Salivary Gland Neoplasms
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<thead>
<tr>
<th>Tumor</th>
<th>Translocation</th>
<th>Fusion oncogene</th>
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<tbody>
<tr>
<td>Adenoid cystic carcinoma</td>
<td>t(6;9)(q22-23;p23-24)</td>
<td>MYB-NFIB</td>
</tr>
<tr>
<td>Hyalinizing clear cell adenocarcinoma</td>
<td>t(12;22)(q13;q12)</td>
<td>EWSR1-ATF1</td>
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<tr>
<td>Mammary analogue secretory carcinoma</td>
<td>t(12;15)(p13;q25)</td>
<td>ETV6-NTRK3</td>
</tr>
<tr>
<td>Mucoepidermoid carcinoma</td>
<td>t(11;19)(q12;p13)</td>
<td>MECT1-MAML2</td>
</tr>
<tr>
<td>Pleomorphic adenoma</td>
<td>t(3;8)(p21;q12)</td>
<td>CTNNB1-PLAG1-LIFR-PLAG1</td>
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<td></td>
<td>t(5;8)(p13;q12)</td>
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Table 1: Salivary gland tumors and their respective translocations. Nomenclature: The “t” indicates a translocation while the first set of parentheses identifies the two chromosomes involved in the translocation, and the second set indicates the breakpoints on the chromosome arms. [O’Connor, C. Human chromosome translocations and cancer. Nature Education. 2008;1(1).]
Mucopepidermoid Carcinoma

The most common malignant salivary gland tumor in both adults and children is the mucopepidermoid carcinoma (MEC), which occurs primarily in parotid gland and is composed of admixed mucocytes, epidermoid cells and intermediate cells forming cysts and nests. A t(11;19)(q12;p13) translocation that pairs MECT1 and MAML2 has been identified as an underlying event in the majority of MECs. The MECT1-MAML2 chimeric (fusion) protein is thought to cause disruption of cell cycle and differentiation functions through dysregulation of c-AMP response element-binding (CREB), resulting in tumorigenesis and increased activation of Notch signaling pathways. The presence of this genetic event in MECs is associated with a lower clinical stage and an overall more favorable outcome.

Pleomorphic Adenoma

Pleomorphic adenoma is a benign salivary gland tumor composed of proliferating ductal and myoepithelial elements in a variably myxoid to chondroid background. An infinite number of architectural configurations can be seen. It is by far the most common salivary gland tumor and primarily involves the parotid and minor glands. Although benign, these tumors have a propensity for recurrence if not completely excised.

Pleomorphic adenomas are also characterized by recurrent chromosomal rearrangements, most commonly t(3;8)(p21;q12) which results in promoter swapping between PLAG1, a novel developmentally-regulated zinc finger gene recently characterized as a proto-oncogene, and CTNBN1 which encodes for β-catenin. The activation of PLAG1 is considered a frequent genetic event occurring in all major cytogenetic subgroups of pleomorphic adenomas, and is also demonstrated in certain mesenchymal tumors.

PLAG1 is also involved in the second most common translocation of pleomorphic adenomas, t(5;8)(p13;q12) which again results in increased expression of PLAG1, except under control of the LIFR (leukemia inhibitory factor) promoter region, which is active in a wide variety of fetal and adult tissues, including normal salivary gland tissue. This information has helped reinforce the theory that pleomorphic adenoma cells originate from a single pluripotent cell type capable of differentiation into a variety of somatic phenotypes.

References


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