Adenoid cystic carcinoma (ACC) is a relatively uncommon tumor, with a reported incidence of 4.5 cases per million individuals. The age range for diagnosis is wide, with tumors arising at almost equal incidence in any decade of adulthood. The salivary and lacrimal glands are among the most common sites at which this tumor arises. It is the most common malignancy to arise in the minor salivary glands, and is also one of the most common cancers of the parotid and sublingual salivary glands. ACC also arises in glandular tissue of the nasal passages and tracheobronchial tree. ACC arises less frequently in sites outside of the head and neck, including the breast and vulva.

Histologically, ACC are composed of small basaloid epithelial tumor cells, with small to moderate amounts of cytoplasm. The nuclei tend not to be pleomorphic, and have small or inconspicuous nucleoli. The tumor is composed of cells that exhibit either luminal epithelial differentiation or myoepithelial differentiation, with myoepithelial differentiation predominating. Several growth patterns have been described for ACC. The most common and most characteristic pattern is the tumor cells arranged in variably-sized nests of cells in a cribriform pattern. The cribriform spaces contain hyaline material, variably eosinophilic or basophilic in color. Most carcinomas with such an appearance will secrete mucin, however in ACC the secreted substances are basement membrane constituents, including proteoglycans. A second growth pattern, typically mixed with the cribriform pattern, is a tubular pattern where the tumor infiltrates in separate gland-like groups with single central lumens. The third growth pattern is a solid growth pattern, where tumor cells grow in sheets without lumen formation. This third pattern has been recognized as representing a higher grade of tumor. Szanto et al proposed a grading scheme for ACC, based on the degree of solid growth pattern, illustrated in Figure 1. This grading scheme has reproducibly been associated with poorer prognosis in distinct patient cohorts. That this grading scheme does indeed reflect a form of tumor progression is supported by cases where ACC tumors have displayed increasing solid growth and histologic atypia during the course of tumor recurrence, with increasingly aggressive clinical behavior.
ACC may also rarely undergo dedifferentiation, with a high grade anaplastic carcinoma component arising in a conventional ACC. High grade transformation is characterized by irregular, jagged infiltrating islands of tumor cells or large confluent areas of solid growth, and the background stroma displays a fibrocellular desmoplastic change, which is typically not seen in conventional ACC. The tumor cells display greater atypia, a higher mitotic rate, and usually have more cytoplasm than conventional ACC cases.

The clinical course of conventional ACC is typically a slow but relentlessly progressive one. Although the majority of patients with ACC are alive at 5 years post diagnosis, the majority will go on to die of their disease. Although good local control is usually achieved by resection of the primary tumor, often accompanied by post-operative radiation therapy, late recurrence is common both regionally and at distant sites. Local recurrence is attributed in part due to the proclivity of ACC for perineural invasion. The neurotropism also contributes to the infiltrative nature of this neoplasm, with deep penetration of adjacent tissues.

Figure 1: Histologic types and grades of ACC. **Panel A:** Cribriform pattern, with no solid growth component (Grade 1). **Panel B:** Tubular pattern, with no solid component (Grade 1). **Panel C:** Cribriform growth pattern, with areas of solid growth comprising less than 30% of tumor (Grade 2). **Panel D:** Predominantly solid growth pattern (Grade 3). All figures H&E stain, original magnification 40x.
vital structures of the craniofacial region along major nerve trunks. Because of these clinical features, ACC has been described as “one of the most biologically destructive and unpredictable tumors of the head and neck.” Unlike most carcinomas of the head and neck, ACC seldom metastasizes to regional lymph nodes. Distant metastasis occurs in up to 40% of cases, with the lungs being the most common site, but liver, kidney, bones and brain also affected by metastatic disease. Due to the slow growth rate of this tumor, even patients with unresectable metastases may survive for many years before ultimately succumbing to their disease. ACC with high grade transformation, however, show a markedly more aggressive clinical course, with shortened patient survival and have a higher rate of lymph node metastasis than conventional ACC.

Several cytogenetic studies of ACC described a reciprocal t(6;9) translocation occurring in some tumors. However it was not clear initially if this was a common finding in ACC, and the described breakpoints of the t(6;9) translocations did not appear to be consistent enough to map specific gene loci. This field of inquiry lay fallow until work from Goran Stenman’s laboratory was published in 2009 which described an analysis showing that the majority of ACC tumors harbor a t(6;9) translocation that join the MYB and NFIB transcription factors in a fusion gene product. The MYB gene appears to be the dominant contributor to the fusion product, with the DNA-binding and transcriptional regulatory portions of the MYB gene on chromosome 6 (6q22-q23) being fused to a small portion of the end of the NFIB gene on chromosome 9 (9p24.1). Though the molecular mechanisms are not entirely elucidated, this fusion gene appears to result in increased MYB transcriptional regulatory activity in the tumor cells. The levels of MYB gene product are generally elevated in ACC compared to most normal tissues, with the majority of ACC showing strong nuclear staining for MYB by immunohistochemistry (Figure 2A).

Figure 2: Examples of MYB alterations in ACC. Panel A: Immunohistochemical stain for MYB showing strong diffuse nuclear staining. Original magnification 100x. Panel B: Break-apart FISH analysis for MYB with 5’ green probe and 3’ red probe. The ACC tumor cell reveals one wild type MYB gene locus (yellow signal) and evidence of break-apart of the second MYB gene locus, with separate red and green signals. Original magnification 1500x.
Though there is some disagreement in the literature regarding the percent of ACC tumors harboring this molecular alteration, most subsequent studies have shown that the majority of ACC have a disrupted MYB gene and/or evidence of a MYB-NFIB fusion RNA transcript \(^{32-36}\). One of the largest independent cohorts separate from the Stenman group came from a study at Stanford University, where 37 cases of ACC were studied by fluorescence in situ hybridization (FISH) of the MYB and NFIB loci, with 65% of cases showing abnormalities of these loci \(^{34}\). An example of an ACC tumor displaying typical FISH findings for a broken-apart MYB gene locus is shown in Figure 2B. One research group showed evidence that some ACC tumors which did not contain a translocated MYB gene had elevated levels of wild-type MYB transcript and protein through an unknown mechanism \(^{32}\). While as previously noted the majority of ACC will show strong MYB immunoreactivity, this stain cannot be used to exclude the diagnosis of ACC, as some tumors (up to a third of cases) that otherwise meet the histologic diagnosis of ACC may show weak or negative MYB staining \(^{34,36,37}\). MYB staining is also not specific for ACC, being observed in several other tumor types, including basaloid squamous cell carcinoma, an occasional histologic mimic of ACC \(^{34,36}\).

Thus it would appear that up-regulation of MYB transcriptional regulatory activity is a key component of tumorigenicity for the majority of, but not all, ACC tumors. Secondary genetic mechanisms are not well understood however. In attempts to identify genomic alterations that occur in ACC, there have been at least 6 studies of ACC cohorts employing the techniques of comparative genomic hybridization (CGH) and array comparative genomic hybridization (aCGH) which globally sample the copy number status of tumor genomes \(^{38-43}\). The results of these studies have been quite variable but areas of deletion that have been identified in two or more tumors in each of two or more of these studies include 1p (8-44%), 5q (8-18%), 6q (14-30%), 9p (12-33%), 9q (8-9%), 12q (9-33%), 13q (11-35%), 14q (4-22%), 17p (11-13%), Xp (8-9%). Chromosomal areas of copy number gain that have been identified in two or more tumors in each of two or more of these studies include 1p (8-44%), 8q (9-39%), 11q (9-61%), 16p (8-44%), 16q (8-39%), 17q 18-28%), 19p (9-78%), 22q (8-72%). It is important to note that none of these studies segregated the ACC samples into MYB fusion positive and negative cases, hence it is unknown if any of the described genetic abnormalities are associated positively or negatively with MYB gene locus translocation. It is also important to note that these techniques only identify changes in chromosomal copy number and will not detect reciprocal translocation events that are not associated with genetic deletion or amplification. Though there is some agreement on loci that show loss or low-level gain in a minority of ACC cases, evidence is generally lacking for specific genes that are targeted by these copy number variations identified by these studies, other than the deletions identified at 6q and 9p, which may be related to MYB-NFIB translocations events that were not perfectly reciprocal in genetic exchange. The studies are in agreement that very few high copy number gene amplification events were
identified in any of the cohorts, hence it does not appear that such a mechanism targets a specific gene in the majority of ACC.

When specific genes have been analyzed in ACC (outside of MYB), there has also been a distressing lack of consensus in the percent of ACC cases having mutations. For instance, analysis of the KIT gene has had studies leading to diametrically opposed conclusions as to the mutation rate in ACC \(^{44-50}\). However, at the current time, the consensus data does not identify any molecular alteration outside of the MYB gene that represents a common molecular mechanism in ACC. This is an active subject of research, and it is expected that planned and ongoing whole exome and whole genome sequencing studies of ACC cohorts will soon contribute to our knowledge in this area.

While secondary genetic hits remain to be elucidated in ACC, there does appear to be a consensus finding that the degree of genetic abnormality present in an ACC tumor is correlated with histologic grade. Analyses of chromosomal ploidy have shown that most classic non-solid ACC show a low degree of aneuploidy, much less than other types of carcinomas, but, ACC with solid histology show increased aneuploidy \(^{51,52}\). Studies using CGH and aCGH show a correlation of the degree of genetic alterations (change in DNA copy number, chromosome number, genetic deletion) with the degree of solid growth pattern \(^{39,41,42}\), consistent with the notion that ACCs with solid histology represent a later stage in tumor progression. One study of four ACC with high grade transformation suggests that genomic amplification events are more common in transformed tumors than in conventional tumors, with identification of the MYC gene as perhaps a common mechanism for transformation \(^{53}\).

Most carcinomas are thought to derive their abnormal genetic patterns from a process known as fusion-breakage-fusion, where unstable telomeres lead to a reiterative process of chromosomal fusion and breakage during subsequent mitotic cycles \(^{54}\). However tumors which occur through a mechanism where a translocation event provides a primary driver mutation tend to have lower degrees of genetic abnormalities. A model is thus developing of the molecular pathogenesis of adenoid cystic carcinoma. It would appear that most ACC begin as low-grade neoplasms, driven to neoplastic growth predominantly by genetic alteration of the MYB locus, with few if any secondary genetic hits. As these tumors continue to grow, eventually the tumors acquire secondary genetic hits, perhaps through a mechanism of telomere shortening and instability. Genetic instability is associated with increasing histologic grade and increased clinical aggressivity, again presumably through involvement of other genetic loci that are involved in more typical and common forms of carcinoma. It may also be the case that these secondary mutations are diverse across the population of higher-grade ACC, hence these tumors may not represent as uniform a genetic and biochemical population as low-grade ACC tumors.
In conclusion, recent findings indicate that up-regulation of the MYB proto-oncogene occurs in the majority of ACC, with translocation and gene fusion of the MYB and NFIB genes being the most common mechanism. The immediate future of research in this field of the molecular pathogenesis of ACC will be twofold. One will be of diagnostic import, determining what combination of molecular assays (FISH, RT-PCR) is the most robust, sensitive and specific for identifying MYB/NFIB genetic alterations in the diagnosis and classification of ACC and related tumors. The second is determining the biochemical and biological affects of MYB activation in ACC tumor cells, to derive targeted therapies for this neoplasm, which is often highly resistant to currently-available conventional therapies.

References

8. Prasad AR, Savera AT, Gown AM, Zarbo RJ: The myoepithelial immunophenotype in 135 benign and malignant salivary gland tumors other than pleomorphic adenoma, Archives of Pathology & Laboratory Medicine 1999, 123:801-806


44. Moskaluk CA, Frierson HF, Jr., El-Naggar AK, Futreal PA: C-kit gene mutations in adenoid cystic carcinoma are rare, Mod Pathol 2010, 23:905-906; author reply 906-907


MYB activation and other genomic alterations in Adenoid Cystic Carcinoma

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Adenoid cystic carcinoma: Clinical features

- **Incidence:** 4/10^6, 500-600/yr U.S.
- **Gender:** M:F 2:3
- **Age:** any time in adulthood
- **Sites:**
  - **Head & neck**
    - Major salivary glands
    - Minor salivary glands
      - Palate, oropharynx
    - Nasal sinuses
    - Lacrimal gland
    - Larynx
  - Trachea & bronchial tree
  - Breast
  - Vulva (Bartholin’s gland)
  - Ear canal (ceruminous glands)
  - Skin (malignant cylindroma?)
Adenoid cystic carcinoma: Clinical features

- **Good 5 year survival**
  - DOD>5yrs p dx common

- **Recurrence is common**
  - Local
  - Distant metastasis

- **Pattern of metastasis**
  - Regional LN mets rare
  - Lung mets most common

- **Highly resistant to Rad Rx and chemotherapy**

Review of 160 pts at M.D. Anderson Cancer Center
Adenoid cystic carcinoma: Histologic variants

- Cribriform
- Tubular
- Solid
Adenoid cystic carcinoma: Histologic grade

Grade 1
no solid component

Grade 2
<30% solid

Grade 3
>30% solid

Histologic grade can change in recurrences & metastases of ACC

- Documented in 3 cases series
  - Marsh WL & Allen MS. Cancer 1979; 43:1463-73
- Rates of progression varied widely among series
  - 29% to 87%
- *Always* progresses to more solid histology
- Suggests that solid histology represents a more advanced tumor progression stage, not merely a morphologic variant
High grade transformation of ACC
(Dedifferentiated ACC)

- Several documented cases where a high-grade carcinoma emerges from ACC

Range of features
- Pleomorphism (nuclear enlargement, prominent nucleoli, increased cytoplasm)
- Increased mitoses
- Necrosis
- Loss of hyaline stroma / BM secretion
- Desmoplastic stroma
- Raggedly infiltrating sheets / nests
High grade transformation of ACC (Dedifferentiated ACC)

- Anaplastic and sarcomatoid transformation also described
- HG transformation associated with rapidly progressive clinical course
  - Death < 3 years
- Lymph node metastases common

Chromosomal translocation

- t(6;9) translocations and chr 6 deletions had been reported in cytogenetic studies of ACC for several decades
  - Incidence and breakpoint specificity initially not well established
  - Uncertainty if these findings were consistent with oncogene activation or tumor suppressor gene deletion
t(6;9) translocation: MYB-NFIB fusion gene

In 2009 Göran Stenman’s laboratory reported that ACC have translocations that fuse the MYB gene (Ch. 6) to the NFIB gene (Ch. 9)

- 11/11 (100%) were found to have the fusion transcript by RT-PCR

Persson M et al. PNAS 106:18740-44, 2009
t(6;9) translocation: MYB-NFIB fusion gene

MYB
DNA binding transactivation negative regulation

NFIB
DNA binding transactivation

MYB-NFIB fusions
MYB gene translocation is correlated with higher levels of MYB gene product

- Most ACC have elevated levels of MYB mRNA and protein compared to normal salivary glands
What is the incidence of the MYB-NFIB translocation in ACC?

- A range of incidence reported in literature
  - 28 – 100%

- Issues: detection method
  - RT-PCR
  - FISH
MYB assays

FISH

WT

BA

RT-PCR

MYB

NFIB
What is the incidence of the MYB-NFIB translocation in ACC?

斯坦福：AJSP (2011) 35:92-99
- 24/37（65%）MYB FISH 异常
  -- 18/37（49%）MYB-NFIB 转位

UVA：未公开数据
- 11/15（73%）MYB FISH 异常
  -- 10/15（67%）MYB-NFIB 转位
MYB gene translocation is correlated with higher levels of MYB gene product.
Are there other common genetic alterations in ACC?

- **Global genetic analysis**
  - Comparative Genomic Hybridization (CGH)
  - Microarrays (aCGH)

- **Gene-specific mutational analysis**
  - PCR-directed sequencing
Are there other common genetic alterations in ACC?

- **Global genetic analysis**
  - CGH and aCGH generally have shown poor consensus between studies in terms of specifically affected loci
  - Consensus that there are no common high copy number gene amplification events
**Are there other common genetic alterations in ACC?**

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<th>Gains (low level)</th>
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<td>1p (8-44%)</td>
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<td>16p (8-44%)</td>
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<td>22q (8-72%)</td>
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</tbody>
</table>

Freier K Cancer Gen Cytogen 2005, 159:89-95
Are there other common genetic alterations in ACC?

◆ Gene-specific mutational analysis
  - Often contradictory results
    - P53, KIT
  - Bottom line: no validated individual gene mutation (outside of MYB-NFIB) found in a substantial fraction of ACC
Increasing genetic abnormalities are associated with increasing histologic grade.

Array CGH data

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<th>Tumor grade</th>
<th>Av. # losses</th>
<th>Av. # bases lost (Mb)</th>
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<tbody>
<tr>
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<tr>
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<tr>
<td>3</td>
<td>34</td>
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</table>

Lab Invest 87:430-435, 2007
Model for ACC tumor progression

Benign glandular epithelial progenitor cell

Initiating genetic event: Myb activation

“Typical” low grade ACC, cribriform & tubular types – stable genomes

Telomere crisis? P53 abnormalities?

Solid (Grade 3) ACC, dedifferentiated ACC – Chromosome instability

Unique ACC phenotype, primarily driven by MYB

Changes “typical” of carcinomas
Conclusions

- t(6;9) occurs in the majority of ACC,
  - results in MYB-NFIB fusion transcripts
  - Results in elevated MYB protein levels
  - Activation of MYB-driven gene expression
    regulation is presumed pathogenic mechanism

- Histologic grading of ACC on the basis of solid growth pattern is correlated with increasing genomic abnormalities
  - Increased clinical aggressiveness of Grade 3 & transformed ACC is likely due to secondary genetic hits