Adenoid Cystic Carcinoma of the Lacrimal Gland: MYB Gene Activation, Genomic Imbalances, and Clinical Characteristics

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Purpose: To investigate genetic alterations in lacrimal gland adenoid cystic carcinomas (ACCs) with emphasis on the MYB-NFIB fusion oncogene and its downstream targets, MYB rearrangements, and copy number alterations in relation to clinical data and survival.

Design: Experimental study.

Participants and Controls: Fourteen patients with primary lacrimal gland ACC were included. As a control, we also studied the expression of MYB-NFIB in 19 non-ACC lacrimal gland tumors.

Methods: The expression and identity of MYB-NFIB fusion transcripts were studied using reverse transcriptase polymerase chain reaction (RT-PCR) and nucleotide sequence analyses. Quantitative polymerase chain reaction (PCR) and immunohistochemistry were used to evaluate the expression of MYB/MYB-NFIB target genes. High-resolution array-based comparative genomic hybridization (arrayCGH) and fluorescence in situ hybridization were used to study copy number alterations and MYB rearrangements.

Main Outcome Measures: mRNA or protein expression of MYB-NFIB, MYB, and its downstream targets; copy number alterations; and genomic rearrangements.

Results: The median age of the patients was 43 years (equal gender distribution), and the median time of survival was 8.6 years. The MYB-NFIB fusion was expressed in 7 of 14 ACCs. In contrast, all non-ACC tumors were fusion-negative. All 13 ACCs tested stained positive for the MYB protein, and for the MYB targets KIT and BCL2, 12 were positive for MYC and CCNE1, and 9 were positive for CCNB1. Rearrangements of MYB were detected in 8 of 13 cases, including 2 cases with gain of an apparently intact MYB gene. The arrayCGH analysis revealed recurrent copy number alterations with losses involving 6q23-q27, 12q12-q14.1, and 17p13.3-p12, and gains involving 19q12, 19q13.31-qter, 8q24.13-q24.21, 11q12.3-q14.1, and 6q23.3. Neither MYB-NFIB fusion nor any copy number alteration correlated with survival.

Conclusions: Lacrimal gland ACCs are frequently positive for the MYB-NFIB fusion, overexpress MYB and its downstream targets, and have genomic profiles characterized by losses involving 6q, 12q, and 17p, and gains involving 19q, 8q, and 11q. Our findings show that lacrimal gland ACCs are genetically and clinically similar to their salivary gland counterparts and that MYB-NFIB is a clinically useful diagnostic biomarker for ACC. Our data also suggest that MYB and its downstream targets are potential therapeutic targets for these tumors.

Financial Disclosure(s): The author(s) have no proprietary or commercial interest in any materials discussed in this article. Ophthalmology 2013;119:1–9 © 2013 by the American Academy of Ophthalmology.
MYB oncogene and of critical MYB target genes associated with cell growth, apoptosis, transcriptional regulation, and cell cycle control. Recent studies have also demonstrated that MYB may be activated by other mechanisms than gene fusion, thus further emphasizing the significance of MYB activation in ACC. Taken together, these studies identify MYB-NFIB/MYB as a new therapeutic target in ACC. In addition to activation of MYB, salivary ACCs also are characterized by recurrent genomic imbalances, including losses involving 1p, 5q, 6q, 11q, 12q, and 14q, and gains involving 1q and 22q. However, the target genes of these rearrangements are still unknown. Recent studies have demonstrated that breast and salivary gland ACCs have different genomic profiles in line with the well-known differences in biological behavior between these tumor types.

Next to nothing is known about MYB-NFIB and other genetic changes in lacrimal ACC, and there are no high-resolution array-based comparative genomic hybridization (arrayCGH) studies performed that permit comparisons with ACCs at other anatomic sites. Therefore, the purpose of this study was to investigate the expression of MYB-NFIB, MYB, and their downstream target genes, as well as to study genomic imbalances and MYB rearrangements in a series of 14 primary lacrimal ACCs and to correlate the findings with clinical data and survival.

Materials and Methods

Patient Data and Tumor Material

We have previously identified all lacrimal gland ACCs diagnosed in Denmark between 1974 and 2007, classified the tumors according to the latest guidelines from the World Health Organization, and staged the tumors according to the 7th edition of the American Joint Committee on Cancer classification. Fourteen of these cases from which sufficient clinical data and tumor material were available were included in the present study. Formalin-fixed paraffin-embedded (FFPE) tumor material was available from all cases, and additional fresh-frozen material was available from 2 cases. The tumors were graded histopathologically, as suggested by Szanto et al., as tumors with no solid component (grade I), tumors with less than 30% solid component (grade II), and tumors with more than 30% solid component (grade III). For controls, we included FFPE tumor material from 2 cases. The tumors were graded histopathologically, as suggested by Szanto et al., as tumors with no solid component (grade I), tumors with less than 30% solid component (grade II), and tumors with more than 30% solid component (grade III). For controls, we included FFPE tumor material from 2 cases.

Reverse Transcription Polymerase Chain Reaction and Nucleotide Sequencing Analyses

Total RNA was extracted from five 10-μm sections obtained from paraffin blocks of 14 ACCs and 19 non-ACC lacrimal tumors using the RNeasy FFPE kit (Qiagen, Hilden, Germany). In addition, total RNA was extracted from 2 fresh-frozen ACC samples (cases 10 and 11) using the RNeasy Mini kit (Qiagen). DNase-treated (DNA-free, Ambion, Austin, TX) total RNA was subsequently converted to cDNA using the iScript cDNA Synthesis Kit (Bio-Rad, Munich, Germany) according to the manufacturer’s manual. As a control for intact RNA and cDNA, reverse transcriptase-polymerase chain reaction (RT-PCR) for expression of ACTB (β-actin) were performed on all samples.

The MYB-NFIB fusion transcripts were amplified by polymerase chain reaction (PCR) as previously described. All tumors were screened for expression of the most common MYB-NFIB fusion transcript variants, including MYB exon 12 or 14 fused to NFIB exons 8a, 8c, or 9. The MYB and NFIB exons were numbered as described previously. As positive controls, ACCs with known MYB-NFIB fusion transcript variants were used. Each PCR reaction was performed in triplicate. The PCR products were gel-purified and sequenced using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA). The resulting sequences were analyzed using the BLAST tool provided by the National Center for Biotechnology Information (available at: http://www.ncbi.nlm.nih.gov; accessed January 16, 2012).

Quantitative Real-Time Polymerase Chain Reaction Analysis

Quantitative PCR (qPCR) analysis was performed on frozen tumor samples (cases 10 and 11) using the AB 7500 Fast Real-time PCR system (Applied Biosystems) as previously described. MYB expression was analyzed using the TaqMan Gene Expression assay for MYB exons 1-2 (Hs00920554_m1) (Applied Biosystems). All samples were assayed in triplicate. The relative expression levels of MYB in tumor and 4 normal salivary gland samples were calculated with the SDS Software v2.0.1 (Applied Biosystems) using the comparative Ct method with the housekeeping gene GAPDH (Hs99999905-m1) as endogenous controls and cDNA from normal salivary gland tissue as calibrator.

Immunohistochemistry

Five-micrometer FFPE sections were treated as previously described using the DAKO EnVision+ System (Dako, Glostrup, Denmark). A Pre-Treatment Module (Dako) with heat-induced epitope retrieval was used for 20 minutes at 97°C in an acid (pH 6.0) or alkaline (pH 9.0) buffer. After blocking of endogenous peroxidase activity with Dako EnVision Flex+ System (Dako, Glostrup, Denmark), the reaction was visualized using the Dako EnVision Flex+ System (Dako, Glostrup, Denmark).

Array Comparative Genomic Hybridization Analysis

Genomic DNA was isolated from FFPE tumor specimens using the Qiagen DNeasy Blood & Tissue Kit (Qiagen) as described for FFPE material (Agilent Technologies, Palo Alto, CA). Sufficient DNA was obtained from 10 of the 14 ACCs. The arrayCGH analysis was subsequently performed on these cases using Human Genome CGH Microarray 244K oligonucleotide arrays (G4411B;
Agilent Technologies). The experiments were performed essentially as recommended by the manufacturer.22,23 Slides were scanned on an Agilent High-Resolution C Microarray Scanner, followed by data extraction and normalization using Feature Extraction v.10.7.1 (Agilent Technologies) with linear normalization (protocol CGH_107_Sep09). Data analysis was carried out using NEXUS Copy Number v.6.0 Discovery Edition (BioDiscovery, El Segundo, CA). The significance threshold for segmentation was set to \( P = 1.0E-6 \), and the settings for aberration calls for all but 1 sample were 1.0 for amplification, 0.4 for gain, −0.4 for loss, and −1.0 for homozygous deletion. For case 10, the settings for aberration calls were 1.0 for amplification, 0.5 for gain, −0.5 for loss, and −1.0 for homozygous deletion. Each aberration was checked manually to confirm the accuracy of the call. A copy number alteration was considered recurrent if 2 or more tumor samples (≥20%) carried the same alteration with a \( P \) value <0.05. Sex chromosomes were excluded from the analysis, as were regions partially or completely covered by a previously reported copy number variation.24,25

**Fluorescence In Situ Hybridization Analysis**

Fluorescence in situ hybridization analysis (FISH) analysis for detection of MYB gene rearrangements were performed on 5-µm FFPE sections using a dual-color MYB split FISH probe (Abnova, Taipei City, Taiwan). The protocols for pretreatment, hybridization, and post-hybridization washes were essentially as recommended by the manufacturer. Fluorescence signals were digitized, processed, and analyzed using the CytoVision image analysis system (Applied Imaging, Newcastle-Upon-Tyne, UK). At least 50 nuclei were scored from each case.

**Results**

**Clinical and Histopathologic Observations**

Of the 14 patients, 7 were men and 7 were women (Table 1). The median age at diagnosis was 43 years (range, 23–67 years). The median duration of symptoms before seeing an ophthalmologist was 6 months (range, 1–24 months), and the most common symptoms were pain (10/14), displacement of the eye (9/14), and decreased eye motility (8/14) (Fig 1A and B, available at [http://aaojournal.org](http://aaojournal.org)). Tumor resection via a lateral orbitotomy was performed in 11 patients, and an orbital exenteration was performed in 3 patients. Eleven patients received postoperative adjuvant radiotherapy. In 8 patients, the tumor had invaded the adjacent structures in the orbit (T4). Histopathologically, 5 tumors were classified as grade I (Fig 1C, available at [http://aaojournal.org](http://aaojournal.org)), 7 tumors were classified as grade II, and 2 tumors were classified as grade III (Table 1). The median time of follow-up was 7 years (range, 1–27 years), and the median tumor-specific survival was 8.6 years. Five patients had local recurrences in the orbit, and in 4 of these the pathology report indicated positive resection margins. Five patients (36%) were still alive at the end of the study period, whereas 8 had died of tumor-related causes (57%), and 1 had died of other causes (7%) (Table 1). The median survival for patients with T4 tumors was 10.3 years compared with 9.8 years for patients with non-T4 disease (\( P = 0.28 \)). Patients with tumors larger than 2.5 cm had a median survival of 8.6 years versus 15.0 years for patients with tumors smaller than 2.5 cm (\( P = 0.28 \)). Tumor grade, T stage, and type of treatment did not correlate with survival. We found no correlation between the MYB-NFIB fusion and the tumor size (<2.5 cm or >2.5 cm).

**Expression of MYB-NFIB Fusion Transcripts**

To study the expression of the MYB-NFIB fusion, we screened all 14 lacrimal ACCs and 19 non-ACC lacrimal neoplasms by RT-PCR (Table 1). One or more chimeric MYB-NFIB transcript variants were detected in 7 of the 14 tumors (Fig 2A). The identities of the chimeric transcript variants identified in both frozen and FFPE tumor samples were verified by nucleotide sequence analysis (Fig 2B). The composition of all fusion transcripts was in agreement with previously published data.24,25 In contrast, none of the 19 non-ACC lacrimal neoplasms expressed MYB-NFIB fusion transcripts. There was no correlation between fusion status and survival.

**Expression of MYB mRNA and Protein and MYB Downstream Targets**

To study the expression of MYB mRNA, we performed qPCR analysis on fresh-frozen tumor tissue from the fusion-positive cases 10 and 11. Both cases overexpressed MYB mRNA compared with normal salivary gland tissue. The expression levels were approximately 10 times (case 10) and 150 times (case 11) higher compared with normal salivary gland tissue (data not shown). In addition, immunohistochemical staining revealed strong nuclear staining of the MYB protein in all 13 lacrimal ACCs tested (Table 1 and Fig 1D ([Fig 1 available at [http://aaojournal.org](http://aaojournal.org)]). To study the molecular consequences of constitutive high-level expression of MYB, we also analyzed the expression of 5 known MYB target genes by immunohistochemistry. All 13 tumors stained positive for KIT and BCL2 (Fig 1E, available at [http://aaojournal.org](http://aaojournal.org)), 12 tumors were positive for MYC and CCNE1, and 9 tumors were positive for CCNB1 (Fig 1F, available at [http://aaojournal.org](http://aaojournal.org)).

**Copy Number Alterations**

Genome-wide arrayCGH analysis using 244K arrays revealed genomic imbalances in all 10 lacrimal gland ACCs analyzed. A total of 44 partial genomic imbalances and 9 gains of whole chromosomes were detected (Fig 3A and Tables 1 and 2 ([Table 2 available at [http://aaojournal.org](http://aaojournal.org)]). The average number of copy number alterations per tumor was 5.1 (range, 1–15). There was no correlation between the number of copy number alterations and the tumor grade. Eleven recurrent copy number changes, defined as minimal common regions of deletions and gains, were identified (Table 3). Recurrent copy number losses were slightly more common than gains (6 vs. 5). The most frequent copy number losses involved 4 different regions within 6q23.3 to 6q27 varying in size from 787 kb to 10.4 Mb (Table 3). These deletions were detected in 3 cases each. Other recurrent losses involved 12q12-q14.1 (3 cases) (Fig 3B) and 17p13.3-p12 (2 cases). The most frequent copy number gains were 19q12 (4 cases), 19q13.31-pter (4 cases), 8q24.13-q24.21 (3 cases), 11q12.3-q14.1 (2 cases), and 6q23.3 (2 cases) (Fig 3C–E). The 2 latter cases showed gain of 1 or more copies of an apparently intact MYB gene (cases 3 and 7). One tumor (case 10) had a breakpoint within the NFIB gene. The only recurrent whole chromosome alterations were gains of chromosomes 19 and 22 seen in 3 tumors each. None of the recurrent copy number alterations correlated with survival.

**Rearrangements of the MYB Locus**

To identify possible rearrangements of the MYB locus, we performed FISH analysis of FFPE sections using a dual-color MYB break-apart probe (Fig 4A, available at [http://aaojournal.org](http://aaojournal.org)). Rearrangements of MYB were detected in 8 of 13 analyzed cases.
Five of these cases also expressed MYB-NFIB fusion transcripts, and 3 were fusion-negative (Table 1). The patterns of MYB rearrangements observed included split signals in 6 tumors (Fig 4B and C, available at http://aaojournal.org) and 2 cases with gain of 1 or more copies of MYB. One of the latter cases showed, in addition to 2 fused green/red signals, gain of 1 or 2 green signals, indicating a selective gain of the MYB locus (Fig 4D, available at http://aaojournal.org). This was also confirmed by arrayCGH showing gain of a 236-kb region including MYB (Fig 3E).

**Table 1. Clinical Data, MYB-NFIB Fusion Status, MYB Gene Rearrangement/Gain, MYB Protein Expression, and Genomic Imbalances in 14 Lacrimal Gland Adenoid Cystic Carcinomas**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yrs)/Sex</th>
<th>Grade</th>
<th>TNM*</th>
<th>Follow-up (yrs)</th>
<th>MYB-NFIB Fusion Status</th>
<th>MYB R/G</th>
<th>MYB Protein Expression</th>
<th>Genomic Imbalances</th>
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<tbody>
<tr>
<td>1</td>
<td>41/M</td>
<td>I</td>
<td>T4b</td>
<td>DM (4), TRD (5)</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>3q13.13</td>
</tr>
<tr>
<td>2</td>
<td>67/M</td>
<td>II</td>
<td>T4c</td>
<td>DOC (3)</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>56/F</td>
<td>I</td>
<td>T4†</td>
<td>LR (2), TRD (12)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>5q13.2-q21.1, 6q21-q23.3, 8q24.13-q24.1</td>
</tr>
<tr>
<td>4</td>
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<td>II</td>
<td>T4c†</td>
<td>DM (1), TRD (1)</td>
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<td>+</td>
<td>+</td>
<td>1q32.1-qter, 6q23.3-qter</td>
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<tr>
<td>5</td>
<td>26/M</td>
<td>I</td>
<td>T4b</td>
<td>DM (4), TRD (4)</td>
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<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>66/M</td>
<td>III</td>
<td>T2†</td>
<td>DM (5), TRD (9)</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>6pter-p22.2, 11, 19</td>
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<td>7</td>
<td>32/F</td>
<td>II</td>
<td>T2†</td>
<td>NED (14)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>5p13.2-q21.1, 5q13.3-qter, 6q24.3-qter, 17p11.2-q25.3, 19</td>
</tr>
<tr>
<td>8</td>
<td>57/F</td>
<td>II</td>
<td>T2†</td>
<td>LR+DM (5), TRD (6)</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>17, 19, 22</td>
</tr>
<tr>
<td>9</td>
<td>23/F</td>
<td>II</td>
<td>T4a†</td>
<td>LR (4), DM (5), TRD (5)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>8, 11q12.3-q14.1, 12p, 12q14, 1-qter, 17q1</td>
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<tr>
<td>10</td>
<td>41/M</td>
<td>I</td>
<td>T3†</td>
<td>LR (2), NED (7)</td>
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<td>+</td>
<td>+</td>
<td>5p11.1-q21.2, 12q12-q14.1, 17p13.3-p12, 12q12-q15</td>
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<tr>
<td>11</td>
<td>41/M</td>
<td>II</td>
<td>T2†</td>
<td>LR (3), NED (8)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>12q11-q14.1, 15q23-q24.1, 15q25.1</td>
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<tr>
<td>12</td>
<td>24/F</td>
<td>III</td>
<td>T4a†</td>
<td>DM (2), TRD (15)</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>13</td>
<td>64/F</td>
<td>II</td>
<td>T4a†</td>
<td>NED (27)</td>
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<td>−</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>14</td>
<td>67/F</td>
<td>I</td>
<td>T2†</td>
<td>NED (7)</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>ND</td>
</tr>
</tbody>
</table>

DM = distant metastases; DOC = dead of other causes; LR = local recurrence; NED = no evidence of disease; NDA = no data available; R/G = rearrangement/gain; TNM = tumor, node, and metastasis; TRD = tumor-related death.

*None of the patients had lymph node or distant metastases at the time of diagnosis.
†Tumor size <2.5 cm.
‡Tumor size >2.5 cm.
§Tumor size not known.

We present the first comprehensive molecular/clinical investigation of lacrimal gland ACC. By using RT-PCR, FISH, immunohistochemistry, and arrayCGH, we have characterized a series of 14 primary lacrimal gland ACCs and correlated the findings with different clinical parameters. Expression of the MYB-NFIB gene fusion was detected in 7 of 14 (50%) lacrimal gland ACCs. This figure is in agreement with recently reported data by Brill et al from a large series of FFPE salivary gland ACCs, as well as from a few lacrimal gland ACCs (1 of 3 analyzed tumors were fusion-positive). The generally lower frequency of fusion-positive ACCs detected in FFPE tumor samples compared with frozen tumor samples (>85% fusion-positivity) is most likely a consequence of degradation and chemical cross-linking of RNA, which is known to occur during formalin fixation and paraffin embedding. In contrast, none of the non-ACC lacrimal neoplasms was fusion-positive, thus confirming that MYB-NFIB is specific also for lacrimal gland ACC. There was no correlation between patient survival and MYB-NFIB status. This may not be surprising because MYB activation by gene fusion or other mechanisms has been shown to occur in more than 90% of ACCs.

To further characterize the status of MYB in lacrimal gland ACC, we performed FISH and immunohistochemical...
Figure 2. Reverse transcription polymerase chain reaction (PCR) analysis of MYB-NFIB fusion transcripts in 14 lacrimal gland adenoid cystic carcinomas (ACCs). A, Detection of MYB-NFIB fusion transcripts in Formalin-fixed paraffin-embedded (FFPE) tumor tissues from 7 of 14 ACCs. ACTB was used as an internal control to test for intact RNA and cDNA. The sizes of the amplified fragments are indicated. B, Nucleotide sequence analysis of the PCR product generated from case 3 confirmed that it corresponded to a chimeric transcript consisting of MYB exon 14 fused to NFIB exon 8c.

Figure 3. The arrayCGH analysis of 10 lacrimal gland adenoid cystic carcinomas (ACCs). A, Genome-wide frequency plot of all copy number alterations detected in the 10 tumors. A total of 27 gains (blue) and 26 losses (red) were identified across the whole genome (sex chromosomes excluded). B–E, The arrayCGH profiles of lacrimal gland ACCs demonstrating (B) loss of 12q11-q14.1 (case 12), (C) gain of 8q24.13-q24.1 including the MYC locus (case 3), (D) gain of 11q12.3-q14.1 including the CCND1 gene (case 9), and (E) gain of 6q23.3 including an apparently intact MYB gene (case 7).
analyses. By using an MYB break-apart probe, we detected MYB rearrangements in 8 of 13 tumors tested, 3 of which were fusion-negative by RT-PCR. This brings the frequency of tumors with MYB rearrangements or MYB-NFIB fusion up to 77%. The pattern of MYB rearrangements detected by FISH was consistent with translocation/gene fusion in 6 cases and with gain of 1 or more copies of MYB in 2 cases. Immunohistochemical staining revealed high nuclear expression of MYB protein in all 13 cases analyzed. The high expression of MYB protein was also confirmed at the RNA level by qPCR analysis of fresh-frozen tumor samples from 2 ACCs. Both cases expressed high levels of MYB mRNA compared with normal salivary gland tissue. Taken together, these findings further emphasize the significance of MYB as a diagnostic biomarker for ACC irrespective of anatomic location.9–12,26

To study the molecular consequences of MYB activation in lacrimal gland ACC, we analyzed the expression of the known MYB/MYB-NFIB targets MYC, BCL2, KIT, CCNB1, and CCNE1, which are involved in critical cellular processes, such as cell growth, apoptosis, transcriptional regulation, and cell cycle control. Immunostaining revealed that all 13 tumors available for analysis overexpressed KIT and BCL2. 12 tumors overexpressed MYC and CCNE1, and 9 tumors overexpressed CCNB1. These findings, which are in line with our previous observation of overexpression of KIT, BCL2, MYC, and CCNB1 at the mRNA level in salivary gland ACC with MYB activation,9 are the first to demonstrate overexpression of these genes also at the protein level, the only exception being KIT, which has been shown to be overexpressed in several previous studies of ACC.17,27 Collectively, these findings clearly indicate that MYB and its downstream targets are activated in lacrimal gland ACC and that they are potential therapeutic targets for these tumors.

By using high-resolution arrayCGH analysis, we explored copy number alterations in 10 lacrimal gland ACCs. Similar to salivary gland ACCs,12 the lacrimal gland counterparts are genetically rather stable with comparably few copy number alterations per tumor (5.1 copy number alteration/tumor in lacrimal ACC vs. 7.7 in salivary ACC) and with no evidence of gene amplification or homozygous deletions. The frequency and spectrum of genomic imbalances are also similar and include recurrent losses involving multiple regions within the distal part of 6q and proximal parts of 12q and 17p.13 The 6q deletions are most likely associated with the ACC-specific (6;9) translocation primarily resulting in the MYB-NFIB gene fusion.13 We found 2 cases with breakpoints close to MYB and with gain of 1 of more apparently intact copies of MYB (both confirmed by FISH). One of these had a small gain of a 236-kb segment harboring only the MYB locus. This case is similar to the genomic duplications of MYB seen in a subset of T-cell acute lymphoblastic leukemia that result in overexpression of MYB.28 To our knowledge, these are the first confirmed examples of selective gain of MYB in ACC. Although the main target of the 6q rearrangements is MYB, we cannot exclude the possibility that the concurrent deletion of 6q sequences in a subset of tumors involve loss/inactivation of 1 or more candidate tumor-suppressor genes in 6q24.2-q26, for example, LATS1, PLAGL1, or PARK2.29–32 Likewise, the 12q and 17p deletions seen in both lacrimal and salivary ACCs might involve loss of tumor-suppressor genes. There are at least 2 candidate tumor suppressor genes located within 12q12-14, and 1 candidate tumor suppressor gene within 17p13.3-p12, that is, LIMA1 and NR4A1 on 12q33–35 and TP53 on 17p.36 Our findings strengthen the notion that these deletions are characteristic of ACC regardless of anatomic location.

The most common copy number gains recorded in the present series of ACC involved 19q12 (4 cases), 19q13.31-qter (4 cases), 8q24.13-q24.21 (3 cases), and 11q12.3-q14.1 (2 cases). There are several oncogenes and cancer-associated genes located within these regions, notably CCNE1, FOSB, RELB, and BCL3 in 19q12-qter13,37–41; MYC in 8q24;42 and CCND1 in 11q13.37,43 Of note, MYC has been shown to be overexpressed in ACC compared with normal salivary gland tissue,44 and gain of 8q24, including MYC, was recently suggested to be associated with high-grade transformation of ACC.45 Likewise, CCND1 has been shown to be both gained46 and overexpressed in
However, additional studies are needed before firm conclusions can be drawn about the role of these and other gained/lost genes in ACC.

The present series of patients with ACC clinically showed similar characteristics with regard to age, gender distribution, symptoms, treatment, and survival as previously reported for patients with lacrimal ACC. However, a relatively large rate of local recurrences (5/14) was observed, and this is most likely because of the lack of clear resection margins in 4 of the cases; all tumors resected via a lateral orbitotomy received postoperative radiotherapy. Only 1 of the cases (case 3) that recurred locally had no evidence of tumor cells in the resection margins, and this case was treated with an orbital exenteration, including removal of the adjacent bone. However, because of the clear resection margins, it was at the time considered unnecessary to administer adjuvant radiotherapy to this patient. Skinner et al. recently emphasized the importance of postoperative radiotherapy for patients with lacrimal gland carcinomas, and our case 3 further illustrates this point.

We observed a relatively large number of T4 tumors (8/14) in our material. The TNM (tumor, node, and metastasis) classification has recently been updated, and the T designation changed from being mainly dependent on tumor size to include, in the case of T4 tumors, all cases with perioseal or cortical bone involvement. This upstaging was recently questioned in a study of 18 consecutive patients with lacrimal gland ACC showing that the change in T designation leads to a higher number of T4 tumors, and as a consequence the correlation between T stage and survival that applied for the old TNM version is lost. Although no significant correlation was found among T stage, tumor size, and survival in the present series, we observed longer median survival times for patients with smaller tumors compared to patients with tumors larger than 2.5 cm (8.6 vs. 15.0 years, \( P = 0.28 \)). This trend did not apply for T stage and survival; of the patients with T4 tumors, 6 of 8 (75%) died of tumor-related causes and 4 of 6 (66%) who did not have T4 disease were still alive without evidence of disease. However, only 1 of these patients had follow-up of 14 years, and the rest had 8 years or less. The current TNM classification needs further validation on larger datasets before any clear conclusions can be drawn as to the correlation with survival.

In conclusion, our molecular and clinical study of lacrimal gland ACC shows that the tumors in most respects are similar to their salivary gland counterparts. Thus, they are frequently positive for the MYB-NFIB fusion; over-express MYB and its downstream targets involved in cell growth, apoptosis, transcriptional regulation, and cell cycle control; and have genomic profiles characterized by in particular losses involving 6q, 12q, and 17p, and gains involving 19q, 8q, and 11q. The fact that the MYB-NFIB fusion is found in a high frequency of ACCs irrespective of anatomic location suggests that it might be a “driver mutation” in this tumor type and that MYB and its downstream targets are novel candidates for therapeutic intervention in the majority of these tumors.

Acknowledgments. The authors thank Ywonne Andrén in the Stenman laboratory for advice regarding qPCR analysis.

References

Footnotes and Financial Disclosures

Originally received: September 17, 2012.
Final revision: March 22, 2013.
Accepted: March 22, 2013.
Available online: Manuscript no. 2012-1420.

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Financial Disclosure(s):
The author(s) have no proprietary or commercial interest in any materials discussed in this article.

Funding: The Danish Cancer Society, The Danish Eye Research Foundation, Fight for Sight Denmark, The Danish Eye Health Society, Synoptik-Fonden, Købmand Kristian Kjær and wife Magrethe Kjær’s Foundation, Kleinsmed Svend Helge Arvid Schröder and wife Ketty Lydia Larsen Schröder’s Foundation, DMSc Alfred Helsted and wife DMSc Eli Møller’s Foundation, Engineer August Fredrik Wedell Erichsen’s Foundation, the Swedish Cancer Society, and BioCARE, a National Strategic Research Program at University of Gothenburg. The funding organizations had no role in the design or conduct of this research.

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The following material should appear online only: Figures 1 and 4, and Table 2.