Human Pathology (2014) **45**, 2270–2280

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

MYB-NFIB gene fusion in adenoid cystic carcinoma of the breast with special focus paid to the solid variant with basaloid features☆

Timothy M. D'Alfonso MD[a,*], Juan Miguel Mosquera MD[a,b], Theresa Y. MacDonald BS[a], Jessica Padilla MA[b], Yi-Fang Liu BS[a], Mark A. Rubin MD[a,b], Sandra J. Shin MD[a]

[a]*Department of Pathology and Laboratory Medicine, Weill Cornell Medical College, New York, NY 10065*
[b]*Institute for Precision Medicine of Weill Cornell Medical College and New York-Presbyterian Hospital, New York, NY 10065*

Received 3 June 2014; revised 15 July 2014; accepted 23 July 2014

Keywords:
Adenoid cystic carcinoma;
MYB-NFIB;
Breast;
Solid variant with basaloid features;
Fluorescence in situ hybridization (FISH)

Summary Adenoid cystic carcinomas (ACCs) from various anatomical sites harbor a translocation t(6;9)(q22-23;p23-24), resulting in *MYB-NFIB* gene fusion. This gene fusion is not well studied in mammary ACCs, and there are no studies examining this abnormality in solid variant of ACC with basaloid features (SBACC), a high-grade variant thought to behave more aggressively than ACCs with conventional histologic growth. Our aim was to investigate the frequency of *MYB-NFIB* gene fusion in mammary ACCs with a focus paid to SBACC. *MYB* rearrangement and *MYB-NFIB* fusion were assessed by fluorescence in situ hybridization and reverse-transcription polymerase chain reaction, respectively. Histologic features and the presence of *MYB* rearrangement were correlated with clinical outcome. *MYB* rearrangement was present in 7 (22.6%) of 31 mammary ACCs (5/15 [33.3%] ACCs with conventional growth; 2/16 [12.5%] SBACCs). One patient with conventional ACC developed distant metastasis, and no patients had axillary lymph node involvement by ACC (mean follow-up, 34 months; range, 12-84 months). Two patients with SBACC had axillary lymph node involvement at initial surgery, and 2 additional patients experienced disease recurrence (1 local, 1 distant; mean follow-up, 50 months; range, 9-192 months). *MYB-NFIB* fusion status did not correlate with clinical outcome in studied patients. We confirm that *MYB-NFIB* gene fusion is observed in mammary ACCs and that a subset lacks this abnormality. This study is the first to confirm the presence of *MYB* rearrangement in SBACC. Additional validation with long-term follow-up is needed to determine the relationship, if any, between *MYB-NFIB* gene fusion and clinical outcome.

1. Introduction

Adenoid cystic carcinoma (ACC) is a malignant tumor composed of epithelial and myoepithelial cells that occurs in a variety of sites including salivary gland, tracheobronchial tree, vulva, and breast. ACC constitutes less than 0.1% of all invasive mammary carcinomas [1-3] and is classified as triple-negative and basal-like carcinomas [4,5], a heterogeneous and typically aggressive subgroup of breast cancers. In contrast to its salivary gland counterpart, which shows poor long-term outcomes, mammary ACC shows low rates of recurrence, and affected patients experience excellent long-term survival [1,2,6,7]. A variant of mammary ACC referred to as the solid variant of ACC with basaloid features

☆ Disclosures: The authors have nothing to disclose.

* Corresponding author. New York-Presbyterian Hospital/Weill Cornell Medical College, 525 East 68th St, Starr 1031E, New York, NY 10065.
E-mail address: tid9007@med.cornell.edu (T. M. D'Alfonso).

http://dx.doi.org/10.1016/j.humpath.2014.07.013
0046-8177/

(SBACC) is a high-grade tumor thought to be associated with a more aggressive clinical course [8-10].

The *MYB-NFIB* fusion gene, resulting from translocation t(6;9)(q22-23;p23-24) involving *MYB* on chromosome 6q and *NFIB* on chromosome 9p, has been identified as a cytogenetic abnormality specific to ACC from various sites [11,12]. The presence of *MYB-NFIB* translocation/gene fusion in ACC of the breast has been studied in small series of patients with reported frequencies of finding *MYB* alteration ranging from 38% to 100% [4,11-14]. No reports have studied *MYB-NFIB* gene fusion in the subset of ACCs classified as SBACC. We studied a large series of mammary ACCs to (i) determine the frequency of finding *MYB* rearrangements in these tumors; (ii) compare the relationship of *MYB* rearrangement with tumor growth pattern and histologic grade, with a focus paid to cases classified as SBACC; and (iii) compare clinicopathological features and the presence of *MYB* rearrangements with clinical outcome.

2. Materials and methods

2.1. Case selection

Thirty-one cases of mammary ACC were identified from our surgical pathology and breast pathology consultation files. The study included 5 cases of SBACC previously reported by Shin and Rosen [8]. Slides and histologic material in the form of formalin-fixed, paraffin-embedded (FFPE) blocks and unstained slides were retrieved. In 3 cases, histologic material was available from needle core biopsy samples, whereas the remaining were from excision specimens. Slides were reviewed by 2 breast pathologists (T. M. D. and S. J. S.) to confirm the diagnosis and select blocks to be used for fluorescence in situ hybridization (FISH) and reverse-transcription polymerase chain reaction (RT-PCR). Tumors were graded according to the system proposed by Ro et al [15] for ACC and by the Elston-Ellis modification of the Scarff-Bloom-Richardson (MBR) system [16]. Growth pattern (cribriform, tubular, solid, basaloid, reticular) was recorded in each case. Clinical data were obtained from electronic records or referring clinicians or pathologists. The study was conducted under an institutional review board–approved protocol.

2.2. FISH analysis

FISH was performed on 5-μm-thick paraffin sections from selected blocks. To detect *MYB* rearrangement, a dual-color break-apart interphase FISH assay was performed using centromeric (BAC clone RP11-349 J5, red) and telomeric (BAC clone RP11-641019, green) probes. In 2 cases, *MYB-NFIB* gene fusion was assessed using a dual-color fusion FISH assay using *MYB* centromeric (BAC clone RP11-349 J5, red) and *NFIB* telomeric (BAC clone RP11-452 J2, green) probes. Tissue pretreatment and probe labeling were performed as previously described [17]. Chromosomal locations of BAC probes were validated on normal metaphase chromosomes. *MYB break apart assay*: Nuclei with a positive *MYB* break-apart signal showed nonoverlapping single centromeric (red) and telomeric (green) signals in 1 allele of tumor cells, whereas the other allele (not rearranged) showed 1 yellow signal, due to superimposed red and green signals. *MYB-NFIB fusion assay*: Using locus specific probes for the 2 genes of interest, nuclei with the gene fusion showed 1 yellow signal, indicating fusion of red (*MYB* locus) and green (*NFIB* locus) signals, and 2 separate red and green signals, which correspond to the other 2 (not fused) alleles. At least 100 nuclei were evaluated per tissue section using a fluorescence microscope (Olympus BX51; Olympus Optical, Tokyo, Japan). Tumors with greater than or equal to 10% abnormal cells were considered positive for rearrangement [4].

2.3. RT-PCR analysis

RT-PCR was performed to detect the presence of the *MYB-NFIB* fusion transcripts in 29 tumors. Total RNA was extracted from FFPE sections using the RNeasy FFPE Kit (Qiagen, Valencia, CA). Complementary DNA was when synthesized using SuperScript III Reverse Transcriptase (Invitrogen, Carlsbad, CA) and run on the ABI7500 to detect fusion transcripts. Each polymerase chain reaction was run in duplicate with a no-template control performed for each run. All tumors were screened for the most common *MYB-NFIB* fusion transcript variants, including *MYB* exon 14 linked to *NFIB* exons 8c, 9, and 8a [11] as well as additional chimeric fusion transcripts, which have previously been described [18] (Table 1).

2.4. Immunohistochemistry

Immunohistochemical staining for estrogen receptor (ER), progesterone receptor (PR), p63, cytokeratin-7 (CK7), and c-kit (CD117) was performed as previously described [19] (Table 2). ER and PR positivity was defined as greater than or equal to 1% of tumor nuclei showing positive staining. For p63, CK7, and c-kit, the pattern and distribution of staining within each tumor were recorded.

3. Results

3.1. Clinicopathological characteristics

The study comprised 31 patients. One group consisted of 15 patients with tumors showing conventional (cribriform-solid, cribriform-tubular) ACC growth patterns (Table 3). The second group consisted of 16 patients whose tumors showed morphologic features of SBACC (described below) (Table 4).