Detecting *MYB* and *MYBL1* Fusion Genes in Tracheobronchial Adenoid Cystic Carcinoma by Targeted RNA-Sequencing

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Abstract

Primary tracheobronchial adenoid cystic carcinoma is rare, accounting for less than 1% of all lung tumors. Many adenoid cystic carcinomas have been reported to have a specific chromosome translocation t(6;9)/*MYB-NFIB*. More recently, t(8;9)/*MYBL1-NFIB* gene fusion was reported in salivary gland adenoid cystic carcinomas which lacked a t(6;9)/*MYB-NFIB*. Two prior studies showed t(6;9)/*MYB-NFIB* in tracheobronchial adenoid cystic carcinoma; however, only rare cases of *MYBL1* rearrangement have been reported in this carcinoma. In this study, we used targeted RNA sequencing to investigate fusion genes in tracheobronchial adenoid cystic carcinoma at our institution. Fusions of either *MYB* or *MYBL1* genes were detected in 7 of 7 carcinomas. Three cases had *MYB-NFIB*, and 3 had *MYBL1-NFIB*. The remaining case showed a rare *MYBL1-RAD51B* fusion. These findings suggest that rearrangement involving *MYB* or *MYBL1* is a hallmark of tracheobronchial adenoid cystic carcinoma.

Keywords

Tracheobronchial Adenoid Cystic Carcinoma; lung; *MYB-NFIB*, *MYBL1-NFIB*, *MYBL1-RAD51B*; RNA sequencing

Primary tracheobronchial adenoid cystic carcinoma is the second most common salivary gland-type carcinoma of the lung after mucoepidermoid carcinoma (1–3). It is rare, accounting for less than 1% of all lung tumors (1, 4, 5). The reported age of adenoid cystic carcinoma patients ranges from 21 to 76 years, with an equal gender distribution (2, 4). Tracheobronchial adenoid cystic carcinoma generally has a protracted clinical course, with...
Multiple recurrences and late metastases. Advanced stage, the presence of positive margins, and a solid histologic pattern are associated with poor prognosis (4). Tracheobronchial adenoid cystic carcinoma has similar pathological features to adenoid cystic carcinoma identified at other anatomical sites, such as: tubular, cribriform, and solid architectural patterns with a variably myxoid/hyalinized basement membrane-like extracellular matrix. The tumor is composed of two different cell types: ductal and myoepithelial cells (4, 5).

Recent genomic studies of adenoid cystic carcinoma have demonstrated a low mutation frequency (6–8). However, 23% to 92% of adenoid cystic carcinoma from the head/neck and breast have been reported to have a specific chromosome translocation t(6;9)(q22-q23;p23-p24)/MYB-NFIB, resulting in the fusion of MYB protooncogene and the transcription factor gene NFIB (5, 9–14). This fusion has been shown to be highly specific for adenoid cystic carcinoma (10) at multiple anatomical locations, including the breast (13, 15), lacrimal glands (16) and skin (17–19). More recently, two groups independently identified a MYBL1-NFIB gene fusion resulting from a (8;9) translocation in salivary gland adenoid cystic carcinomas which lacked a t(6;9)/MYB-NFIB (8, 20).

Using RT-PCR, Brill et al. identified the t(6;9)/MYB-NFIB in 5 of 10 cases of tracheobronchial adenoid cystic carcinoma (21). In a separate study using fluorescence in situ hybridization (FISH) analysis, Roden et al. identified the t(6;9)/MYB-NFIB in 12 of 29 cases (41%) of pulmonary adenoid cystic carcinoma (5). To date, only rare cases of MYBL1 rearrangement have been reported in tracheobronchial adenoid cystic carcinoma (22).

RNA sequencing can identify multiple fusion genes including new fusion partners in a single assay compared to PCR or FISH tests; which can only detect a limited number of known fusion genes. In this study, we used targeted RNA sequencing to investigate fusion genes in tracheobronchial adenoid cystic carcinoma at our institution. We report fusions of either MYB or MYBL1 genes in 7 of 7 tumors tested, 6 of which involved the fusion partner NFIB. The remaining case showed a rare MYBL1-RAD51B fusion.

Materials and methods

Patients and samples

Seven cases of tracheobronchial adenoid cystic carcinoma diagnosed between 2005 and 2016 were retrieved from the Department of Pathology, Fox Chase Cancer Center. For case 2, a metastasis in the lung was analyzed, as the primary tracheal adenoid cystic carcinoma was not available for testing (Table 1). Pertinent clinical information was collected. This study was approved by the Institutional Review Board at our institution.

RNA-sequencing and data analysis

 Archived formalin-fixed paraffin embedded tumor tissue was used for RNA sequencing. For each tumor, RNA was isolated from five 10-μm thick tissue sections. A High Pure FFPET RNA Isolation Kit (Roche, Indianapolis, IN) was used according to the manufacturer’s protocol. RNA was quantified using a Nanodrop apparatus and evaluated with an Agilent 2100 bioanalyzer.
Next generation sequencing-based targeted RNA-sequencing analysis was performed using the Illumina TruSight RNA Fusion Panel and a MiSeq sequencer according to the manufacturer’s recommendations (Illumina, San Diego, CA) (23). The Trusight RNA fusion panel is a targeted RNA fusion panel that consists of 507 of the most well-known cancer-related fusion partners. This panel covers 7,690 exonic regions that are targeted with a total of 21,283 probes. The gene list is available at www.illumina.com.

**Results**

**Clinical Characteristics**

The adenoid cystic carcinoma cohort consisted of four females and three males ranging in age from 51 to 81 (mean, 63 years) (Table 1). Three of seven cases had a smoking history. For case 1, only a biopsy was available; and the patient received radiation therapy. This patient died 8 years after. All other patients underwent surgical resection; and five of which had clinical follow-up. Within a follow-up period between 2 and 27 years, four patients were alive without disease; and one lived with disease.

**Pathological Findings**

The pathological features are summarized in Table 1. Five tumors were in the trachea, and 2 were from the main bronchus. Tumor size ranged from 1.5 to 6 cm. Four tumors were predominantly cribriform, and 3 cases showed a more tubular formation (Fig 1). All 7 cases (100%) demonstrated fusions involving either MYB or MYBL1, and in 6 of the 7 cases, the fusion partner was NFIB. In one case, however, we identified a fusion of MYBL1-RAD51B.

**Discussion**

Adenoid cystic carcinoma is the second most common malignancy of salivary glands with a poor long-term prognosis (24). The identification of recurring t(6;9)/MYB-NFIB and t(8;9)/MYBL1-NFIB chromosomal rearrangements have significantly enhanced our knowledge of the pathogenesis of adenoid cystic carcinoma (19). MYB is a member of the c-MYB transcription factor family, which also encompasses the structurally related MYBL1 (AMYB) and MYBL2 (BMYB) proteins. The encoded proteins by MYB and MYBL1 genes have a nearly identical DNA binding domain and a similar overall structure (25). The structure of the MYB-NFIB fusion gene is very similar to the MYBL1-NFIB fusion; which preserves the DNA binding and transactivation domains in all fusion proteins. These two fusions are mutually exclusive in adenoid cystic carcinoma (8, 19, 20). In addition, adenoid cystic carcinoma tumors with MYB and MYBL1 fusions display similar gene expression profiles, suggesting that the related MYB proteins are interchangeable oncogenic drivers in adenoid cystic carcinoma (8, 20).

Mitani et al. found the t(8;9)/MYBL1-NFIB in 35% of t(6;9)/MYB-NFIB-negative salivary gland adenoid cystic carcinomas, and all of the MYBL1 alterations they identified involved deletion of the C-terminal negative regulatory domain and were associated with high MYBL1 expression (8). Togashi et al. reported that 97 of 100 cases of head/ neck adenoid cystic carcinoma harbored genomic rearrangements of the MYB (73 cases) or MYBL1 loci (24 cases) (22). Fujii and colleagues found that 29 of 33 (88%) cases of salivary gland
adenoid cystic carcinoma exhibited rearrangements in **MYB**, **MYBL1** or **NFIB** based on FISH analysis (26).

Among tracheobronchial adenoid cystic carcinoma, there have been two other reports on **MYB-NFIB** fusion. In one study, Brill et al. found the **MYB-NFIB** fusion in 50% of cases of tracheobronchial adenoid cystic carcinoma by RT-PCR (21). In the second report, Roden identified the t(6;9)/**MYB-NFIB** in 41% of tracheobronchial/pulmonary adenoid cystic carcinoma by FISH (5).

In this report, we identified fusion genes involving either **MYB** or **MYBL1** in all 7 cases of tracheobronchial adenoid cystic carcinoma, including 3 with **MYB-NFIB**, 3 with **MYBL1-NFIB**, and 1 with a **MYBL1-RAD51B**. Only one previous salivary gland adenoid cystic carcinoma case with a MYBL1-RAD51B fusion gene has been reported, in which an MYBL1 protein truncation occurred due to a translocation between exon 9 of **MYBL1** and intron 5 of the **RAD51B** gene, which resides on chromosome 14 (20). This fusion led to antisense transcription of part of the **RAD51B** intron, such that there was no expression of the RAD51B protein (20).

The **MYB-NFIB** fusion protein is the major mechanism of 5’ MYB up-regulation in adenoid cystic carcinoma, since 3’ MYB contains highly conserved binding sites for certain microRNA molecules, including miR-15a, miR-16 and miR-150. These miRNAs can down regulate 30% of wild-type MYB mRNA (9, 19). Furthermore, fusion genes involving **MYB** and **MYBL1** lose 3’ elements which are responsible for their target specificity. Therefore, the encoded fusion oncoproteins can induce general/non-specific downstream gene expression (27). The exact role of NFIB as a fusion partner remains to be elucidated; although it has been proposed that it may provide stabilizing or regulatory elements to transcription factors such as MYB and MYBL1 (19, 21).

As the genomic hallmark of adenoid cystic carcinoma, MYB and MYBL1 fusion genes can be used for differential diagnosis in routine clinical practice. Concurrently, there is no consensus on the utility of MYB and MYBL1 fusions as prognostic markers (19), although they and their downstream effectors are being investigated as potential therapeutic targets (19, 21). For example, Andersson et al demonstrated that **MYB-NFIB** is an oncogenic driver that can be targeted therapeutically in adenoid cystic carcinoma by inhibiting IGF1R/AKT signaling (28). Moreover, targeting downstream effectors of MYB/MYBL1, such as c-KIT, might provide an alternative approach to treat adenoid cystic carcinoma (19).

**Conclusion**

In this study, seven cases (100%) of tracheobronchial adenoid cystic carcinoma demonstrated translocations involving either **MYB** or **MYBL1** genes; in six of which the fusion partner was **NFIB**. The remaining case showed a fusion **MYBL1-RAD51B** fusion. These findings suggest that rearrangement involving MYB or MYBL1 is a hallmark of this carcinoma. RNA-sequencing studies in a large cohort are warranted to confirm the high frequency of these fusions.
Acknowledgments

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Figure 1.
(a), Tubular-predominant tumor invades bronchial cartilage (case 3). (b), Tubular-predominant tumor (case 7) shows two cell populations: inner ductal cells and the outer myoepithelial cells. (c), Cribriform-predominant tumor with cylindromatous microcystic spaces containing basophilic mucoid material and perineural invasion (case 6). (d), Cribriform tumor with hyalinized basement membrane material (case 5).
### Table 1.
Clinicopathological characteristics of 7 cases of Tracheobronchial adenoid cystic carcinoma

| No. | Sex | Age (Years) | Smoker | Tumor Location | Size (cm) | Histologic Pattern | Gene Fusion | Pathologic Stage | Treatment | Follow-up (Years) |
|-----|-----|-------------|--------|----------------|-----------|-------------------|-------------|------------------|-----------|------------------|
| 1   | M   | 51          | No     | Trachea        | 6.0       | Tubular           | MYB-NFIB   | N/A              | Radiation | 8/DOD            |
| 2   | F   | 68          | No     | Trachea        | N/A ²    | Cribriform        | MYB-NFIB   | N/A              | Surgery   | 27/AWD           |
| 3   | F   | 61          | No     | Trachea        | 4         | Tubular           | MYB-NFIB   | pT2NxM0         | Surgery/radiation | 4/AWOD     |
| 4   | M   | 52          | Yes    | Main bronchus  | 3.5       | Cribriform        | MYBL1-NFIB | pT3N1M0         | Surgery/chemo/radiation | 3          |
| 5   | F   | 81          | Yes    | Trachea        | 2.5       | Cribriform        | MYBL1-NFIB | pT4N0Mx        | Surgery/radiation | 12/AWOD    |
| 6   | F   | 61          | No     | Main bronchus  | 1.5       | Cribriform        | MYBL1-NFIB | pT3N2M0        | Surgery/radiation | 3/AWOD    |
| 7   | M   | 64          | Yes    | Trachea        | 3.2       | Tubular           | MYBL1-RAD51B | pT4N0M0      | Surgery/radiation | 2/AWOD    |

¹Predominant histological pattern,

²Biopsy only, size on CT scan,

³Metastasis,

³Loss of follow-up, DOD, died of disease, AWD, alive with disease, AWOD, alive without disease,