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The Diagnostic Utility of MYB Immunohistochemistry for Adenoid Cystic Carcinoma Arising In External Auditory Canal

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1. ABSTRACT

Adenoid Cystic Carcinoma (ACC) is a rare tumor that grows slowly but often causes distant metastases. The most common site of occurrence is salivary glands, but it can also occur in External Auditory Canal (EAC). There are some reports that MYB Immunohistochemistry (IHC) is useful for the diagnosis of ACC, but there are no reports of ACC arising in EAC diagnosed by MYB IHC. In this study, the usefulness of MYB IHC for the diagnosis of ACC arising in EAC was examined. Four cases of ACC arising in EAC were identified at Gifu University hospital from 2004 to 2021. IHC including MYB was performed on these cases. Then, the staining intensity of MYB was scored based on the previous report. Three of the four cases were positive for MYB IHC. One case was scored "0" and three cases were diagnosed to have a score of "2". S-100, α-SMA, AE1/AE3 and c-kit were positive staining in all cases, and CEA was negative staining in all cases. MYB IHC was performed on 4 cases of ACC arising in EAC. Three of the four cases were positive for MYB IHC and its utility was confirmed.

2. Keywords: Adenoid cystic carcinoma; External auditory canal tumor; Ear canal; MYB; MYB-NFIB; Immunohistochemistry

3. Abbreviations: ACC: Adenoid Cystic Carcinoma; EAC: External Auditory Canal; IHC: Immunohistochemistry; SMA: Smooth Muscle Actin; MSA: Muscle Specific Actin; EMA: Epithelial Membrane Antigen; CEA: Carcinoembryonic Antigen

3. INTRODUCTION

Adenoid Cystic Carcinoma (ACC) is a slow-growing, but relentless malignancy composed of epithelial cells and myoepithelial cells.[1] ACC is a rare tumor that occurs in 2 out of 100,000 people and accounts for less than 1%

of head and neck cancers.[2] The most common site of occurrence is salivary glands, but it can also occur in head and neck region such as oral cavity, sinuses, palate, nasopharynx, lacrimal glands, and External Auditory Canal (EAC).[3] Most external auditory canal tumors are squamous cell carcinomas, and ACC accounting for 5%.[4] ACC arising in EAC is derived from the ceruminous gland.[1] Histologically, this tumor is a mixture of tumor cells that differentiate into myoepithelial and ductal epithelial, and it is classified into three types, cribriform type, tubular type, and solid type, depending on growth pattern of both cells.[3,5] Surgical treatment is the first choice for resectable cases, but standard treatment for unresectable or recurrent metastases has not been established. In 2009, MYB-NFIB fusion gene resulting from the t (6:9) (q22-23; p23-24) translocation was discovered in ACC.[6] There are variations depending on the report, the incidence of MYB-NFIB fusion is ranging from 23% to 86%.[7] Some studies underscored the usefulness of immunohistochemical staining of MYB for ACC diagnosis, and Mitani Y et al.[8] reported that 85% of fusion gene-positive tumors and 61% of fusion gene-negative tumors were positive nuclear staining for MYB [8]. Immunostaining may be better at diagnosing ACC than analyzing fusion genes, as MYB expression may also be mediated by other pathways.[9,10] However, there are no reports of ACC arising in EAC diagnosed by MYB Immunohistochemistry (IHC). In the present study, MYB IHC was performed on four cases of ACC arising in EAC and its utility was examined.

4. MATERIALS AND METHODS

4.1. Patients and sampling

Four cases of ACC arising in EAC were identified at Gifu University hospital from 2004 to 2021. They underwent surgical treatment, and then all the specimens were fixed with buffered formalin, sectioned and stained with hematoxylin and eosin, and confirmed by pathologists based on the 4th edition of WHO classification. The clinical features of the patients are described in Table 1, and the findings of ear canal, Computed Tomography, and Magnetic Resonance Imaging are shown in Figure 1. All relevant clinical data were acquired from patient medical records.

Table 1: Patient characteristics.

Characteristic	Case1	Case2	Case3	Case4
Age	63	77	73	72
Sex	Female	Male	Female	Female
Symptoms	Tragus mass	Ear pain	Ear fullness	Ear canal swelling
Disease duration	48 months	9 months	5 months	2 months
Preoperative pathological diagnosis	Suspicion of ceruminal adenocarcinoma	Suspicion of ACC, basal cell adenoma, adenocarcinoma	ACC	Suspicion of ACC
T classification	T4	T1	T1	T3
Histological type	Cribriform type	Cribriform type	Cribriform type	Solid type
Treatment	Surgery+PORT	Surgery	Surgery	Surgery+PORT
Recurrence (Period after treatment)	+ (53 months)	+ (52 months)	-	-
Observation period	168 months	83 months	4 months	4 months
Outcome	Death	Lost to follow-up	Survival	Survival

ACC: Adenoid Cystic Carcinoma

PORT: Postoperative Radiotherapy

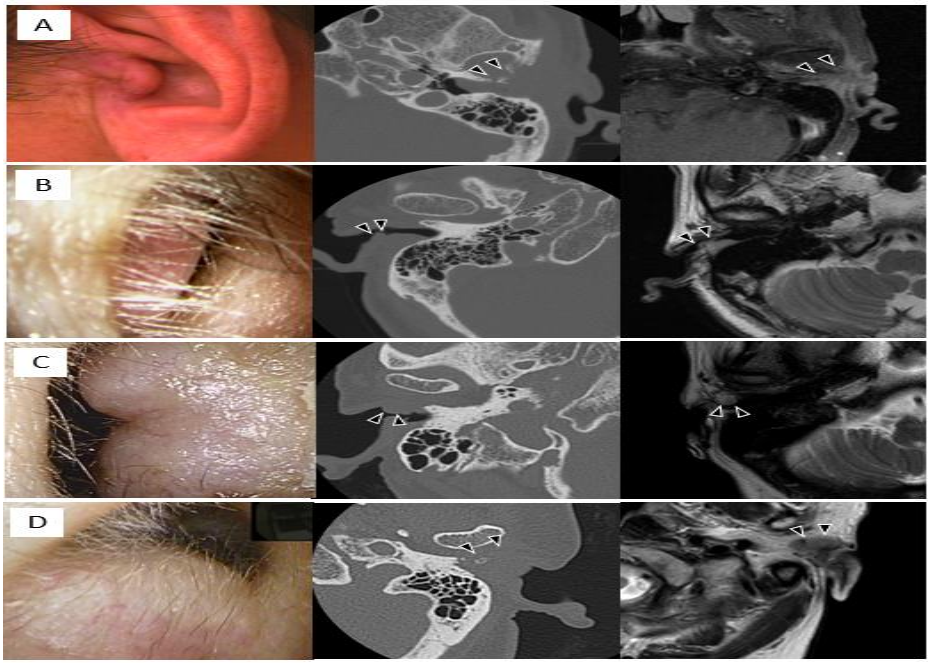

Figure 1: Preoperative findings.

In all cases, a smooth-surfaced mass was found in external auditory canal (EAC). Computed tomography (CT) and magnetic resonance imaging (MRI) showed tumors confined to the ear canal in Case 2 (b) and Case 3 (c) (shown with arrowhead). The tumor invades the anterior wall of EAC in Case 4 (d), and the tumor has extended to the temporomandibular joint in Case 1 (a) (shown with arrowhead).

4.2. Immunohistochemical staining

The immunophenotypic profiles were determined using formalin fixed paraffin embedded specimens. Immunohistochemical staining was performed using automated staining platform Ventana Benchmark Ultra (Roche) and the following primary antibodies were used: MYB, Smooth muscle actin (SMA), Muscle specific actin (MSA), S100, p63, Cytokeratin AE1/AE3, epithelial membrane antigen (EMA), Carcinoembryonic antigen (CEA), c-kit, ki-67. Anti-c-MYB antibody EP769Y (Abcam cat. #45150, RRID: AB_778878) was used for MYB IHC.

4.3. MYB nuclear staining intensity score

The staining intensity of MYB was scored based on the report by Sun T. et al.[10] In brief, scattered to no nuclear staining in tumor cells is scored as "0", pale and focal nuclear staining of < 50% tumor cells is scored as "1", moderate nuclear staining of > 50% tumor cells is scored as "2", diffuse strong staining in tumor cells which easily visible with a low power objective is scored as "3".

5. RESULTS

In Case 1, Case 2, and Case 3, conventional histological examination showed many small cystic cavities found in the tumor cell nest, which is recognized as a cribriform pattern (Figure 2a, b and c). These cases were diagnosed with ACC of cribriform type. In Case 4, tumor cells with a high nuclear/cytoplasmic ratio proliferated solidly, with some comedo-like necrosis (Figure 2d). This case was diagnosed with ACC of solid type.

Three of the four cases were positive for MYB IHC. Case 1 was scored "0" (Figure 3a) and Case 2, Case 3 and Case 4 were judged to have a score of "2" (Figure 3b, c and d).

In other IHC, α-SMA, S-100, AE1/AE3 and c-kit showed positive staining in all cases. MSA, p63 and EMA were positive in three of the four cases, and CEA was negative in all cases (Table 2). The i-67- positive cells were from 10% to 40%.

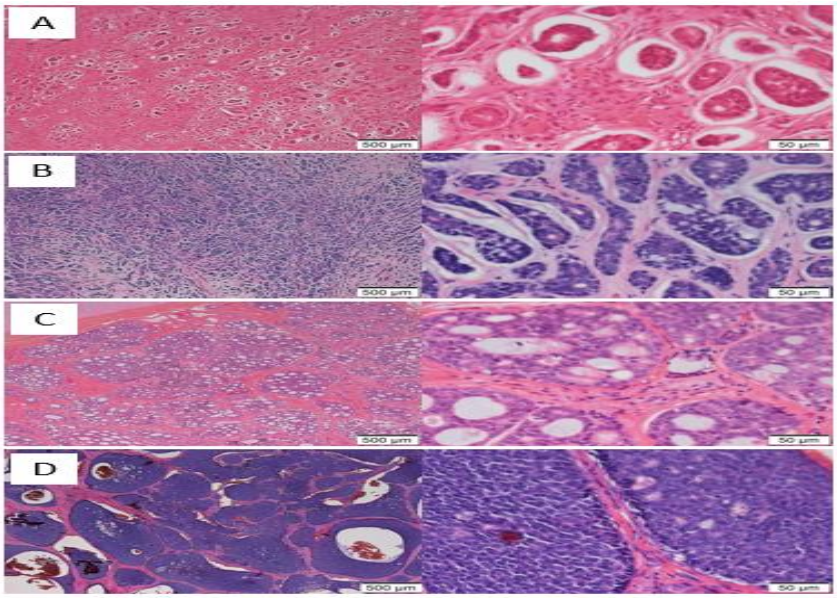

Figure 2: Histopathological findings.

In Case 1 (a), Case 2 (b), and Case 3 (c), many small cystic cavities were found in the tumor cell nest and the tumor cells infiltrated while forming a cribriform pattern. In Case 4 (d), tumor cells with a high N / C ratio proliferated solidly, and this case was diagnosed with adenoid cystic carcinoma of solid type. (Left panel: H & E ×40, Right panel: H & E ×400).

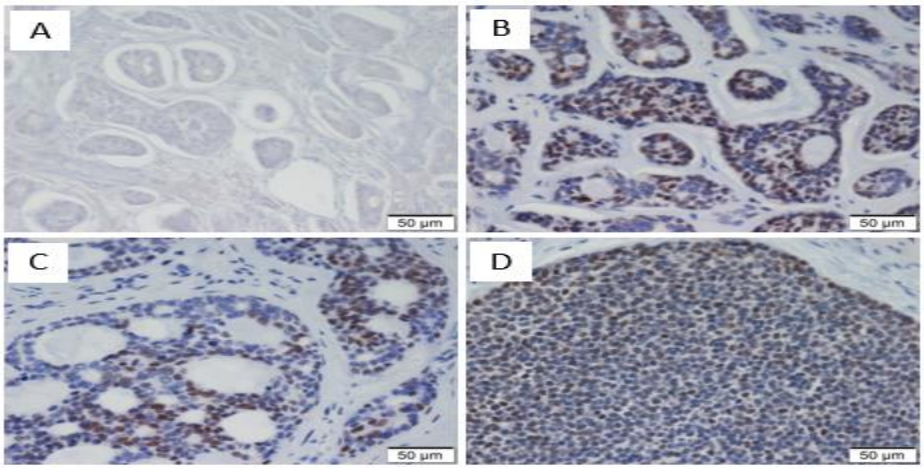

Figure 3: MYB immunohistochemistry.

Three of the four cases were positive for MYB immunohistochemistry. Case 1 (a) was scored "0" and Case 2 (b), Case 3 (c) and Case 4(d) were judged to have a score of "2".

Table 2: Immunohistochemical findings.

Case	MYB	α-SMA	MSA	S-100	p63	AE1/AE3	EMA	CEA	c-kit	ki-67
1	-	+	+	+	-	+	-	-	+	10%
2	+	+	-	+	+	+	+	-	+	10%
3	+	+	+	+	+	+	+	-	+	20%
4	+	+	+	+	+	+	+	-	+	40%

SMA: Smooth muscle actin

MSA: Muscle specific actin

EMA: Epithelial membrane antigen

CEA: Carcinoembryonic antigen

6. DISCUSSION

The fusion gene of MYB-NFIB is a characteristic of ACC and a key oncogenic event in the pathogenesis of ACC.[6] MYB is an oncogene with transcriptional regulatory function and plays an important role in cell proliferation and differentiation, and involved in the carcinogenesis of many types of tumors.[11,12] NFIB is known to encode nuclear transcription factor.[13,14] Overexpression of MYB demonstrated by IHC may be more useful at diagnosing ACC than analyzing the fusion gene, as MYB expression may also be mediated by other pathways without the fusion gene.[9,10,15] In addition to ACC of salivary gland, previous reports have shown that MYB is also expressed in ACC of sinonasal tract, tracheobronchial tree, breast, vulva, and skin.[16] However, there is no report examining the utility of MYB IHC for ACC arising in EAC. In the present study, we investigated the utility of MYB IHC for ACC arising in EAC by analyzing four surgical specimens. 75% of the cases showed positive staining, which was comparable to the results reported in other organs. Preoperative diagnosis of tumor of EAC is very difficult because diseases other than malignancies can often occur in the EAC and the amount of biopsy samples that can be obtained is usually small. Therefore, tissue biopsy is often performed many times. MYB IHC helps to diagnose ACC preoperatively from among tumors of EAC that are difficult to diagnose. However, a score of "0" or "1" is also found in benign or other malignancies, and thus should be interpreted with caution.[10]

In the present study, we also examined the utility of immunohistochemistry other than MYB in ACC arising in EAC. One of the receptor tyrosine kinase, c-kit is characterized for positive in gastrointestinal stromal tumor, mastocytosis, germ cell tumors, acute myeloid leukemia and ACC. And ACC is reported positive for c-kit in 94%.[17] In the present study as well, all 4 cases were positive for c-kit. Previous reports have shown that 67% of ACC are positive for CEA immunohistochemistry, but all cases were negative in our study.[18] This means that the specificity of CEA expression in ACC arising in EAC is low. Thus, IHC including MYB were shown to be more useful in diagnosing ACC arising in EAC.

7. CONCLUSION

MYB IHC was performed on four cases of ACC arising in EAC. Three of the four cases were positive for MYB IHC and its utility was confirmed. We hope to accumulate the number of cases MYB IHC of ACC arising in EAC in the future.

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