Original Article The utility of TLE1 and BCOR as immunohistochemical markers for angiomatoid fibrous histiocytoma

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Abstract: Objectives: Diagnosis of angiomatoid fibrous histiocytoma (AFH) can be challenging due to its variable histologic features and a lack of highly sensitive and/or specific immunohistochemical markers. The utility of TLE1 and BCOR as immunohistochemical markers for AFH is not known. Methods: We examined the spectrum of histologic features of 36 AFHs, and studied the expression of both TLE1 and BCOR in AFH and its mimics by immunohistochemical staining. Positive nuclear expression was scored semiquantitatively. Results: Both typical and unusual histologic features of AFHs were observed in this cohort. TLE1 was moderately to strongly positive in 36/36 AFHs, 4/4 synovial sarcomas, and 2/3 BCOR sarcomas; weakly positive in 4/6 inflammatory myofibroblastic tumors; negative in all dermatofibromas (n = 10), atypical fibrous histiocytomas (n = 5), myofibroma (n = 2) and juvenile xanthogranulomas (n = 5), with an overall sensitivity of 100%, and specificity of 71.4% for AFH. BCOR was moderately to strongly positive in 24/36 AFHs, 4/4 synovial sarcomas, 3/3 BCOR sarcomas, and 1/5 atypical fibrous histiocytomas; weakly positive in 10/36 AFHs; negative in the remaining tumors. The overall sensitivity and specificity of BCOR for AFH were 94.4% and 77.1%, respectively. Conclusions: TLE1 is a highly sensitive immunohistochemical marker for AFH.

Keywords: Angiomatoid fibrous histiocytoma, immunohistochemical staining, TLE1, BCOR, utility, pitfalls

Introduction

Angiomatoid fibrous histiocytoma (AFH) is a rare soft tissue neoplasm, most often arising in the deep dermis and subcutis of the extremities of children and young adults [1]. The current World Health Organization (WHO) classification of bone and soft tissue tumors classifies AFH as a tumor of intermediate malignant potential [2], with an indolent course in general. but with occasional local recurrence (up to 15%) and rare metastasis (<2-5%). Accurate diagnosis of AFH is important for appropriate treatment but sometimes challenging [3-6]. Classical histologic features of AFH include: multinodular growth of histiocytoid/spindle cells, pseudoangiomatous spaces filled with blood, a thick fibrous pseudocapsule, and a peripheral lymphoid "cuff". However, it should be noted that AFH may exhibit a very wide morphologic spectrum [5], including a myxoid variant [7], and more and more unusual sites are being documented [8-12]. Genetically, AFH is characterized by translocations involving EWSR1-CREB1 (about 90%), EWSR1-ATF1 and rare FUS-ATF1 [13]. The identification of these gene fusions is helpful for diagnosis. However, molecular testing is relatively expensive and not universally available.

Currently, no single immunohistochemical (IHC) marker is highly sensitive and/or specific for AFH. IHC markers such as epithelial membrane antigen (EMA), desmin, CD99, and CD68 are being used clinically with variable positivity (50%-75%) and extensive overlap with other diagnostic entities [5, 14]. The majority of AFH cases are negative for S-100, CD21, CD35,

TLE1 in angiomatoid fibrous histiocytoma

| Case | Lymphoid cuffing | Pseudoangiomatous spaces | Fibrous pseudocapsule | Pleomorphism | Myxoid change | Sclerosing change | TLE1 (IHC score) | BCOR (IHC score) | EWSR1 fusion |
|------|---------------------|-----------------------------|--------------------------|--------------|------------------|-------------------|---------------------|---------------------|-----------------|
| 1 | - | + | - | - | - | - | 6 | 6 | NA |
| 2 | + | + | + | + | - | - | 9 | 6 | NA |
| 3 | + | + | + | + | - | + | 9 | 3 | NA |
| 4 | - | + | + | - | - | - | 9 | 9 | NA |
| 5 | - | + | + | - | + | - | 6 | 1 | NA |
| 6 | + | + | + | - | - | + | 9 | 6 | NA |
| 7 | - | + | - | - | - | - | 6 | 6 | NA |
| 8 | + | + | + | - | - | - | 6 | 6 | NA |
| 9 | + | + | + | - | - | + | 9 | 9 | NA |
| 10 | - | + | + | - | - | - | 9 | 9 | NA |
| 11 | + | + | + | - | + | - | 6 | 6 | NA |
| 12 | + | + | + | - | - | - | 6 | 6 | EWSR1+ |
| 13 | - | + | - | - | - | - | 9 | 9 | EWSR1+ |
| 14 | + | + | + | - | - | + | 6 | 6 | EWSR1- and FUS- |
| 15 | + | - | + | - | + | + | 9 | 3 | EWSR1+ |
| 16 | + | + | + | + | - | - | 9 | 6 | EWSR1+ |
| 17 | + | + | + | + | + | - | 9 | 9 | EWSR1-CREB1 |
| 18 | - | + | + | - | - | - | 9 | 6 | EWSR1-CREB1 |
| 19 | + | + | + | - | - | - | 9 | 0 | NA |
| 20 | - | + | - | - | - | - | 6 | 4 | EWSR1+ |
| 21 | - | + | + | - | - | - | 6 | 0 | NA |
| 22 | + | + | + | - | - | - | 6 | 2 | NA |
| 23 | + | + | + | - | - | - | 6 | 6 | EWSR1+ |
| 24 | + | + | + | - | - | + | 6 | 2 | NA |
| 25 | - | + | - | - | - | - | 9 | 9 | EWSR1-ATF1 |
| 26 | + | + | + | - | - | - | 9 | 6 | EWSR1-CREB1 |
| 27 | + | - | + | - | - | - | 6 | 9 | EWSR1-CREB1 |
| 28 | + | - | + | + | + | - | 9 | 4 | NA |
| 29 | + | + | - | - | - | - | 9 | 4 | EWSR1+ |
| 30 | + | + | + | - | - | + | 9 | 4 | EWSR1+ |
| 31 | + | + | - | - | - | - | 9 | 6 | EWSR1+ |
| 32 | - | + | + | + | - | + | 9 | 9 | EWSR1+ |
| 33 | - | + | + | - | - | - | 9 | 9 | NA |
| 34 | + | - | + | - | - | - | 9 | 6 | NA |
| 35 | + | + | + | - | - | - | 9 | 2 | NA |
| 36 | + | + | + | - | - | - | 6 | 6 | EWSR1-CREB1 |

Table 1. Summary of the main histologic features, TLE-1 and BCOR staining and molecular study results in 36 AFH cases

+: positive, -: negative, EWSR1+: an EWSR1 rearrangement was detected by FISH without partner information, NA: Not available.



Figure 1. Representative examples of characteristic architectural patterns and variable cytologic features of AFH. A. The tumor was surrounded by dense lymphoid infiltrates, thick fibrous pseudocapsule, and a small pseudoangiomatous space filled with blood in the center. B. Scanning magnification view of tumor showing a multinodular growth pattern. C. Oval histiocytoid cells with vesicular nuclei and indistinct cell boundaries, admixed with lymphoplasmacytic infiltrates, arranged in a vague whorling. D. Spindled tumor cells arranged in short fascicles. E. Clusters of large epithelioid cells with vesicular nuclei, prominent nucleoli and an alveolar growth pattern, surrounded by abundant lymphoplasmacytic infiltrates. F. Sheets of bland small round tumor cells with scanty cytoplasm and rare mitosis.

cytokeratins, CD31, and CD34 [5]. Additional immunohistochemical markers may be helpful in the evaluation of difficult/atypical cases and in triaging appropriate cases for targeted molecular studies.

Individually, TLE1 and BCOR are often used to identify synovial sarcoma (SS) and BCOR sarcoma; however, they are expressed in other tumors. Recently, we encountered a challenging primary adrenal AFH with lymph node metastases. While evaluating this case, we noted that both TLE1 and BCOR demonstrated strong and diffuse nuclear positivity. Prompted by this experience, we evaluated the expression of both TLE1 and BCOR in AFH and its cutaneous mimics in order to determine their diagnostic value and possible pitfalls.

Materials and methods

Case selection

AFH cases were retrospectively collected from 6 institutions: Children's Hospital Los Angeles (CHLA), Children's Healthcare of Atlanta, Washington University in Saint Louis, University of Oklahoma Health Sciences Center, Cincinnati Children's Hospital and Rhode Island Hospital. In addition, 28 fibrohistiocytic/ myofibroblastic cutaneus tumors/lesions (10 dermatofibromas, including two with an aneurysmal pattern, 6 IMTs, 5 atypical fibrous histiocytomas, 2 myofibromas, and 5 JXGs) along with 4 SS and 3 BCOR sarcomas were collected from CHLA. The hematoxylin and eosin (H&E)-stained slides, as well as available supporting IHC and molecular test results, were re-reviewed by three pathologists (HP, JB, and SZ) to confirm the diagnoses.

Immunohistochemical staining and scoring

Representative whole tissue sections from each of the 36 primary AFH cases and the 35 other lesions/tumors were immunohistochemically stained for TLE1 and BCOR along with appropriate controls using a Leica Bond Max Instrument (Leica, Buffalo Grove, IL). Tissue sections (4 μ m) were deparaffinized and rehydrated using the Leica Bond Max De-Wax solution. Antigen retrieval was performed with Leica Bond ER2 solution for 10 minutes. Then the primary antibodies against TLE1 (1:10, polyclonal; Santa Cruz Biotechnology, Dallas, TX) and BCOR (1:100, C-10; Santa Cruz Biotechnology) was applied and incubated for 15 minutes at ambient temperature. Next the



Figure 2. Examples of unusual histologic features. A. Striking nuclear pleomorphism with large bizarre cells with eosinophilic intranuclear inclusion admixed with eosinophils. B. Sclerosing stroma with scattered large atypical cells surrounded by small round tumor cells. C. Predominant myxoid stroma containing oval histiocytoid small to large cells. D. Pseudoangiomatoid space filled with blood and accompanied by degenerative large bizarre cells.

antibody was detected using the BOND Polymer Refine Detection (Leica), which contains a peroxide block, post primary polymer reagent, DAB chromogen and hematoxylin counterstain.

TLE1 and BCOR nuclear staining were scored semiquantitatively for extent (0, <25%; 1, 25-49%; 2, 50-75%; 3, >75%) and intensity (0-absent; 1-weak; 2-moderate; 3-strong) by two pathologists (HP and SZ), who were blinded to the diagnoses. An IHC composite score (score range: 0-9) combining both extent and intensity was used (positive: \geq 1; weak: <4; moderate: 4-6; strong: >6) for each case. Cases with discordant scores were adjudicated by consensus.

Results

Clinical characteristics of AFH

The detailed clinical information of the 36 AFH cases was reported in a previous study [15]. Briefly, the age range was 2-15.5 years (median age: 8 years) with a female to male ratio of 1.4:1. Primary tumors arose from upper extremities (33%), head and neck (25%), lower extremities (19%), trunk (19%), and adrenal gland (3%).

The size of the tumors ranged from 0.4 to 10.5 cm in greatest dimension. Positive EW-SR1 rearrangement was confirmed in 16/17 tested, including 4 cases with an EWSR1-CREB1 fusion and one case with an EWSR1-ATF1 fusion.

Histologic features of AFH

H&E sections of each case were re-reviewed by three authors (HP, JB and SZ) and the histologic features are shown in Table 1. A total of 19/36 (52.8%) cases exhibited the classical triad (a thick fibrous pseudocapsule, a peripheral lymphoid "cuff", and pseudoangiomatous spaces) (Figure 1A). Twelve cases lacked the typical lymphoid "cuff", 4 cases were without pseudoangiomatous spaces, and 7 cases without a fibrous pseudocapsule. The tumor

cells appeared mostly oval and histiocytoid (Figure 1C), followed by spindle-shaped (Figure 1D), and rarely epithelioid (Figure 1E) and small round cells (Figure 1F). The predominant tumor architecture was a multinodular growth pattern (Figure 1B), although patternless pattern and an alveolar growth pattern (Figure 1D) were recognizable in some cases. Six cases showed striking moderate to marked nuclear pleomorphism with large hyperchromatic nuclei, some with eosinophilic intranuclear inclusions (Figure 2A). Focal prominent sclerosing changes in the stromal component were identified in 8 cases (Figure 2B). Five cases exhibited obvious myxoid change in the stroma of tumor (Figure 2C). Pseudoangiomatous spaces were commonly associated with osteoclastlike/foreign body type giant cells (Figure 2D). Rare mitosis was evident in all cases. No atypical mitotic figures were found in any case. All cases demonstrated variable intratumoral lymphoplasmacytic infiltrates. One tumor showed a focal eosinophilic infiltrate.

TLE1 expression

Immunohistochemical staining results of TLE1 are summarized in **Table 2**. Positive nuclear

| Diagnosis | Overall positivity No./Total | Strong (%) | Moderate (%) | Weak (%) | Negativity (%) |
|-------------------------------|---------------------------------|------------|--------------|----------|----------------|
| AFH | 36/36 | 61 | 39 | 0 | 0 |
| Dermatofibroma | 0/10 | 0 | 0 | 0 | 100 |
| Atypical fibrous histiocytoma | 0/5 | 0 | 0 | 0 | 100 |
| IMT | 4/6 | 0 | 0 | 67 | 23 |
| JXG | 0/5 | 0 | 0 | 0 | 100 |
| Myofibroma | 0/2 | 0 | 0 | 0 | 100 |
| SS | 4/4 | 50 | 50 | 0 | 0 |
| BCOR sarcoma | 2/3 | 66.7 | 0 | 0 | 33.3 |

| Table 2. TLE1 expression in AF | Hs and other tumors |
|--------------------------------|---------------------|
|--------------------------------|---------------------|

AFH: Angiomatoid Fibrous Histiocytoma; IMT: Inflammatory Myofibroblastic Tumor; JXG: Juvenile Xanthogranuloma; SS: Synovial Sarcoma.



Figure 3. Examples of TLE1 and BCOR staining in AFH and its mimics. A-F: TLE1 nuclear staining; strong (A) and moderate (B) staining in AFH, weak staining in inflammatory myofibroblastic tumor (C), negative staining in dermatofibroma (rare staining in endothelial cells) (D), strong staining in synovial sarcoma (E) and BCOR sarcoma (F). G-L: BCOR nuclear staining; strong (G) and moderate (H) staining in AFH, moderate staining in one atypical fibrous histiocytoma (I), negative staining in dermatofibroma (J), moderate staining in synovial sarcoma (K) and strong staining in BCOR sarcoma (L).

TLE1 expression was clearly evident in all AFH cases, with either strong (61% with a composite score of 9) (Figure 3A) or moderate (39% with a composite score of 6) staining (Figure 3B). Interestingly, 4/6 inflammatory myofibroblastic tumors (IMTs) were weakly positive (Figure 3C). There was no immunostaining in any of the dermatofibromas (Figure 3D), atypical fibrous histiocytoma, juvenile xanthogranulomas (JXG) or myofibroma. As expected, SS showed either strong (2/4) (Figure 3E) or mod-

erate (2/4) staining. 2/3 BCOR sarcomas demonstrated strong staining (**Figure 3F**) and the remaining one was completely negative. The overall sensitivity and specificity of TLE1 for AFH among the cases tested were 100% and 71.4%, respectively.

BCOR expression

Immunohistochemical staining results of BCOR are summarized in **Table 3**. BCOR immunoreac-

| Diagnosis | Overall positivity No./Total No | Strong (%) | Moderate (%) | Weak (%) | Negative (%) |
|-------------------------------|------------------------------------|------------|--------------|----------|--------------|
| AFH | 34/36 | 25 | 41.2 | 27.8 | 5 |
| Dermatofibroma | 0/10 | 0 | 0 | 0 | 100 |
| Atypical fibrous histiocytoma | 1/5 | 0 | 20 | | 80 |
| IMT | 0/6 | 0 | 0 | 0 | 100 |
| JXG | 0/5 | 0 | 0 | 0 | 100 |
| Myofibroma | 0/2 | 0 | 0 | 0 | 100 |
| SS | 4/4 | 0 | 100 | 0 | 0 |
| BCOR sarcoma | 3/3 | 66.7 | 33.3 | 0 | 0 |

Table 3. BCOR expression in AFHs and other tumors

AFH: Angiomatoid Fibrous Histiocytoma; IMT: Inflammatory Myofibroblastic Tumor; JXG: Juvenile Xanthogranuloma; SS: Synovial Sarcoma.

tivity was positive in 34/36 AFH cases with 66.2% of cases showing moderate to strong staining (Figure 3G, 3H) and 27.8% of cases showing weak staining. In comparison, BCOR was negative in all dermatofibromas (Figure 3J), IMTs, JXGs and myofibroma, while demonstrating moderate staining in 1/5 atypical fibrous histiocytoma (Figure 3I). All 4 cases of SS exhibited moderate staining (Figure 3K). As expected, all 3 BCOR sarcomas demonstrated strong to moderate staining (Figure 3L). The overall sensitivity and specificity of BCOR for AFH among the cases tested were 94.4% and 77.1%, respectively.

Discussion

The diagnosis of AFH can be challenging when the tumor arises in an unusual anatomic location, shows non-classical histologic features, or in small biopsies where architectural features may not be appreciable [6]. The differential diagnosis includes a heterogeneous group of benign and malignant lesions [16-18]. Due to its rarity and variable histology, analysis of clinicopathologic features and expression of TLE1 and BCOR in AFH may improve its diagnosis. In this study, we observed both typical and unusual histologic features of AFHs and found that the sensitivity of TLE1 and BCOR for AFH were 100% and 94.4%, respectively.

In line with previous observations [7, 17, 19, 20], we found that approximately half of AFH cases exhibited the classic triad characterized by pseudoangiomatous spaces filled with blood, a thick fibrous pseudocapsule, and prominent peripheral lymphoid aggregates with occasional germinal centers. Tumor cell mor-

phology was variable and included histiocytoid, spindle, epithelioid, and small round cells. In addition, unusual histological features such as marked pleomorphism, sclerosing and myxoid stromal changes, and osteoclast-like foreign body type giant cells were noted. We found that these "unusual features" were usually focal and often centrally located, consistent with degenerative changes. Interestingly, an alveolar growth pattern was noted in two cases, which has not been described before.

AFH is categorized by the current WHO Classification of Tumours as having an uncertain histogenesis [2]. No single IHC marker is highly sensitive or highly specific for AFH. TLE1, a highly sensitive IHC marker for SS, was reported to be positive in 7 AFH cases in three different case reports [18, 21, 22]. However, TLE1 has not been tested in a large series of AFH, and possible diagnostic pitfalls have not been well-characterized. We found that TLE1 was moderately to strongly expressed in all 36 AFH cases, meaning that TLE1 is a highly sensitive IHC marker for AFH. Only 4 IMTs of 28 fibrohistiocytic/myofibroblastic cutaneus tumor/lesions showed weak staining. As expected, all SS tumors showed moderate to strong nuclear staining of TLE1. Since AFH and SS may have overlapping histological features and positive CD99 [18], TLE1 positivity represents a significant diagnostic pitfall. When approaching a biopsy specimen with spindle cell morphology and positive staining for TLE-1, CD99 and EMA, it is important to include AFH in the differential diagnosis.

This is the first study to show that BCOR was expressed in the majority of AFHs (34/36) with

variable staining intensity. BCOR was not expressed in dermatofibroma, IMT, JXG or myofibroma. Compared to TLE1, BCOR showed less frequent positivity and overall weaker staining. In addition, one of 5 atypical fibrous histiocytoma showed moderate immunoreactivity for BCOR. As expected [23], BCOR was expressed in all SS and BCOR sarcomas. When facing a challenging AFH case, adding BCOR staining may be helpful, but one should be aware of these pitfalls.

The limitations of our study include the relatively small number of AFH cases and its mimics, its retrospective nature, and that not all AFHs underwent molecular testing. Nevertheless, all our AFH cases and mimics were reviewed by at least 3 pathologists with consensus on diagnosis and all diagnostically challenging AFH cases were supported by molecular data.

In summary, TLE1 is a highly sensitive immunohistochemical marker for AFH. Both TLE1 and BCOR may have diagnostic utility in difficult/ atypical AFH cases in combination with other IHC markers. This study also illustrated a diagnostic pitfall in differentiating AFH from other tumors such as SS and BCOR sarcoma when using these two IHC markers only.

Disclosure of conflict of interest

None.

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