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Angiomatoid fibrous histiocytoma: comparison of fluorescence in situ hybridization and reverse transcription polymerase chain reaction as adjunct diagnostic modalities ☆

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ABSTRACT

Angiomatoid fibrous histiocytoma (AFH) is a rare soft tissue neoplasm of intermediate biologic potential and uncertain differentiation, most often arising in the extremities of children and young adults. Although it has characteristic histologic features of a lymphoid cuff surrounding nodules of ovoid cells with blood-filled cystic cavities, diagnosis is often difficult due to its morphologic heterogeneity and lack of specific immunoprofile. Angiomatoid fibrous histiocytoma is associated with recurrent chromosomal translocations, leading to characteristic *EWSR1-CREB1*, *EWSR1-ATF1*, and, rarely, *FUS-ATF1* gene fusions; fluorescence in situ hybridization (FISH), detecting *EWSR1* or *FUS* rearrangements, and reverse transcription-polymerase chain reaction (RT-PCR) for *EWSR1-CREB1* and *EWSR1-ATF1* fusion transcripts have become routine ancillary tools. We present a large comparative series of FISH and RT-PCR for AFH. Seventeen neoplasms (from 16 patients) histologically diagnosed as AFH were assessed for *EWSR1* rearrangements or *EWSR1-CREB1* and *EWSR1-ATF1* fusion transcripts. All 17 were positive for either FISH or RT-PCR or both. Of 16, 14 (87.5%) had detectable *EWSR1-CREB1* or *EWSR1-ATF1* fusion transcripts by RT-PCR, whereas 13 (76.5%) of 17 had positive *EWSR1* rearrangement with FISH. All 13 of 13 non-AFH control neoplasms failed to show *EWSR1-CREB1* or *EWSR1-ATF1* fusion transcripts, whereas *EWSR1* rearrangement was present in 2 of these 13 cases (which were histopathologically myoepithelial neoplasms). This study shows that *EWSR1-CREB1* or *EWSR1-ATF1* fusions predominate in AFH (supporting previous reports that *FUS* rearrangement is rare in AFH) and that RT-PCR has a comparable detection rate to FISH for AFH. Importantly, cases of AFH can be missed if RT-PCR is not performed in conjunction with FISH, and RT-PCR has the added advantage of specificity, which is crucial, as *EWSR1* rearrangements are present in a variety of neoplasms in the histologic differential diagnosis of AFH, that differ in behavior and treatment.

1. Introduction

Angiomatoid fibrous histiocytoma (AFH) is a rare soft tissue tumor of intermediate (rarely metastasizing) biologic potential and uncertain differentiation. It predominantly arises superficially, in extremity deep dermis or subcutis of children and young adults [1–3]. Angiomatoid fibrous histiocytoma is associated with 3 characteristic chromosomal translocations: t(2;22)(q34;q12) and t(12;22)(q13;q12) (which are not specific for AFH and have been characterized in other classes of neoplasms) and, rarely, t(12;16)(q13;p11), leading to *EWSR1-CREB1*, *EWSR1-ATF1*, and *FUS-ATF1* gene fusions. Although AFH has characteristic histologic features, of nodules of ovoid to spindle cells with blood-filled pseudoangiomatoid spaces and a surrounding lymphoid cuff, diagnosis is often difficult due to significant variation in morphology, histologic overlap with several lesions, lack of specific immunoprofile, and its increasing documentation at unusual extrasomatic sites. Molecular cytogenetic and molecular analyses, for *EWSR1* or *FUS* rearrangements with fluorescence in situ hybridization (FISH), or *EWSR1-CREB1* and *EWSR1-ATF1* fusion transcripts by reverse transcription-polymerase chain reaction (RT-PCR), are therefore useful ancillary diagnostic techniques in the routine setting. In this study, we compared the utility of FISH for detection of *EWSR1* rearrangement and RT-PCR for *EWSR1-CREB1* and *EWSR1-ATF1* fusion transcripts as ancillary tools in the histopathologic diagnosis of AFH.

2. Methods

All cases were formalin fixed and paraffin embedded and comprised consecutive tumor specimens retrieved from the Histopathology

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Indexing System database which were coded as "AFH," over a 6-year period from 2008 to 2014. Cases comprised both core biopsy and excision specimens of material biopsied or resected at our center, and external cases, which had been sent for histologic review or second opinion. Only cases which had FISH or RT-PCR performed for *EWSR1* rearrangement or *EWSR1-CREB1* and *EWSR1-ATF1* fusion transcripts were included. Clinical information was retrieved for each patient from the electronic patient record. All diagnoses had been previously made based on morphology and immunohistochemistry by 1 or both of 2 specialist soft tissue pathologists (KT and CF). In addition, for use as negative controls, cases of neoplasms with a definite non-AFH histopathologic diagnosis, but in which FISH or RT-PCR had been performed to exclude the unlikely scenario of AFH, were retrieved from the molecular and molecular cytogenetics databases (DG and JS). For FISH, 2-μm-thick formalin fixed and paraffin embedded sections were dewaxed overnight at 60°C, treated with hot buffer wash at 80°C (2-3 hours) then proteolytic enzyme treatment at 37°C, and, finally, washed in distilled water and then an alcohol series before addition of an *EWSR1* break-apart probe (Abbott Laboratories Ltd, Maidenhead, UK). Hybridization was performed overnight according to the manufacturer's protocols. Reverse transcription-polymerase chain reaction was performed to assess for *EWSR1-CREB1* and *EWSR1-ATF1* fusion transcripts according to standard or previously described methods [4,5].

3. Results

The group consisted of 7 males and 9 females, with an age range of 8 to 63 years (median, 19 years) (Table). There were 17 specimens in total, from 16 patients (1 patient had 2 excisions of AFH, as a primary tumor of the scalp and as a metastasis to cervical node [cases 5 and 6]). The commonest site was the upper extremity (4 cases), followed by the lower extremity (3 cases), and scalp and lung (2 cases each). Four cases occurred at "unusual" "extrasomatic" sites (2 in lung, 1 in mediastinum, and 1 in meninges). Histologically, all tumors were composed of sheets of ovoid to spindled cells with minimal cellular atypia and showed either or both of an at least partial surrounding lymphoid cuff and pseudoangiomatoid spaces (Fig. 1A-C). No atypical histologic variants were identified. The immunohistochemical features are listed in the Table. Immunohistochemically, desmin was at least focally positive in 10 of 11 tumors. Focal immunopositivity for smooth muscle actin (SMA) was present in 5 of 9 cases, with focal epithelial membrane antigen (EMA) in 5 of 8, focal AE1/AE3 in 3 of 7, at least focal CD68 expression in 2 of 7, and focal CD99 expression in 3 of 8.

All 17 specimens were positive for FISH, RT-PCR, or both, supporting the histopathologic findings and consistent with AFH (Table). *EWSR1* rearrangement was seen with FISH (Fig. 1D) in 13 of 17 samples and was undetectable in 4. Reverse transcription-polymerase chain reaction was performed in 16 samples. Of 16, 14 (87.5%) were shown to harbor either *EWSR1-CREB1* (10 cases) or *EWSR1-ATF1* (4 cases) fusion transcripts (Figs. 2 and 3), and these were mutually exclusive. *EWSR1-CREB1* and *EWSR1-ATF1* fusion transcripts were undetectable in 2 cases, whereas RT-PCR was not performed in 1 case in which there was insufficient material; in all these 3 cases, FISH showed an *EWSR1* rearrangement. Correspondingly, in all 4 specimens where FISH for *EWSR1* rearrangement was negative (which included the primary and metastatic samples from 1 patient), RT-PCR showed the presence of *EWSR1-CREB1* fusion transcripts. Technical success was the same for both, with no fails for either FISH or RT-PCR.

In detecting AFH, the sensitivity of RT-PCR was 87.5%, with specificity of 100%. The positive predictive value of RT-PCR was 100%, and its negative predictive value was 86.7%. The sensitivity of FISH in detecting AFH was 76.5%, with specificity of 84.6%. The positive predictive value of FISH for AFH was 86.7% and negative predictive value, 73.3%.

Of the 2 AFH without detectable *EWSR1-CREB1* or *EWSR1-ATF1* fusion transcripts with RT-PCR, 1 was an excision biopsy and 1 a core biopsy. Of the 4 AFH without detectable *EWSR1* rearrangement with FISH, there were 2 excision specimens and 2 core biopsies. For the 2 cases without detectable *EWSR1-CREB1* or *EWSR1-ATF1* fusion transcripts with RT-PCR, cycle threshold (Ct) values for the control gene (*β-2 microglobulin*) were low (25.6 and 26.7) (Ct, <30 indicating abundant target nucleic acid). For the 4 samples without detectable *EWSR1* rearrangement with FISH but with RT-PCR positivity, the dCt values (ie, the difference between the Ct value for the sequence of interest and the Ct value for the reference (house keeping gene) sequence) for control gene (*β-2 microglobulin*) and fusion gene (*EWSR1-CREB1*) were higher than 8 in 2 cases, which could potentially indicate a low number of cells containing the fusion below the limit of detection of FISH. Nonetheless, there were another 2 cases with dCt values higher than 8 where FISH detected an *EWSR1* rearrangement, indicating that dCt value alone was not enough to estimate the level of fusion-positive cells.

Thirteen cases were used as negative controls. These were benign fibrous histiocytoma, cavernous hemangioma, diffuse type giant cell

Table
Clinical, immunohistochemical, FISH, and RT-PCR findings for AFHs

Case	Age (y) /sex	Site	Desmin	SMA	CD68	CD99	Other IHC	*EWSR1* FISH	RT-PCR *EWSR1-CREB1*	RT-PCR *EWSR1-ATF1*
1	37/F	Inguinal region	+ Focal	NA	NA	NA	NA	+	−	−
2	39/M	Meninges	+ Focal	NA	NA	NA	− myogenin, h-caldesmon, claudin 1	+	−	+
3	63/M	Lung	NA	NA	NA	NA	NA	+	+	−
4	12/M	Axilla	+ Focal	−	+ Diffuse	−	+ Very focal S-100 protein; − AE1/AE3	+	+	−
5	8[a]/M	Scalp	+ Diffuse	−	−	+ Focal	+ Focal EMA, very focal AE1/AE3	−	+	−
6	8[a]/M	Lymph node	+ Diffuse	−	−	+ Focal	+ Focal EMA, very focal AE1/AE3	−	+	−
7	61/M	Lung	+ Focal	−	−	−	+ Focal EMA, calretinin, and AE1/AE3	+	−	+
8	10/F	Chest wall	NA	NA	NA	NA	NA	+	−	+
9	11/F	Knee	+ Focal	+ Focal	−	−	+ Focal EMA	+	+	−
10	55/F	Upper arm	+ Focal	+ Focal	−	−	− EMA and AE1/AE3	+	−	+
11	13/M	Forearm	NA	NA	NA	NA	NA	+	+	−
12	11/F	Elbow	NA	NA	NA	NA	NA	−	+	−
13	16/F	Knee	NA	NA	NA	NA	NA	+	NA	NA
14	17/F	Scalp	NA	NA	NA	NA	NA	−	+	−
15	46/M	Shin	+ Very focal	+ Focal	+ Focal	−	+ Focal CD34, − AE1/AE3, and EMA	+	+	−
16	25/F	Mediastinum	−	+ Focal	NA	NA	+ Very focal CD34, − AE1/AE3, and EMA	+	−	−
17	19/F	Forearm	+ Multifocal	+ Focal	NA	+ Focal	+ Diffuse EMA; − myogenin, h-caldesmon, AE1/AE3, CD34, MUC4, GLUT1, and S-100 protein	+	+	−

Abbreviations: *IHC*, immunohistochemistry; *NA*, not applicable; +, positive; −, negative.
[a] Denotes the same patient, who had both primary and metastatic tumors analyzed.