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COMMUNICATION

Angiomatoid Fibrous Histiocytoma: Pleomorphic Variant Associated with Multiplication of EWSR1-CREB1 Fusion Gene

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Abstract Angiomatoid fibrous histiocytoma (AFH) is a rare soft tissue tumor which exceptionally occurs in visceral organs or bones. Histologically this is a bland, monomorphic tumor and only occasionally shows pleomorphism. Vast majority of the soft tissue cases share the same translocation and the resulting EWSR1-CREB1 gene fusion as background pathogenetic alteration. Here we report a 10-year-old boy with subcutaneous tumor of the right shoulder. Histological, immunohistochemical and FISH analyses of the case revealed pleomorphic phenotype, characteristic immunophenotype and multiplication of the EWSR1-CREB1 fusion gene in the nuclei of the tumor cells. The possible explanation of the fusion gene multiplication, its relation to the morphology and the clinical outcome are discussed in the context of the published literature.

Keywords AFH · Angiomatoid fibrous histiocytoma · Pleomorphic · Multiplication · EWSR1-CREB1 fusion gene

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Introduction

Angiomatoid fibrous histiocytoma (AFH) is a tumor of low malignant potential occurring mainly in soft tissues of children and adolescents [1]. Thirty years ago Enzinger first described as a fibrohistiocytic neoplasm based on the phenotype of the tumor cells [1]. Twelve years later Fletcher demonstrated desmin expression (myoid immunophenotype) in the tumor cells in five of six cases examined [2]. The largest series so far involving 158 cases was published in 1999 by Farnburg-Smith et al. based on the Soft Tissue Registry of the AFIP. In the above study the authors further illustrated the tumor's myoid immunophenotype and suggested a possible myofibroblastic origin [3]. The exact histogenesis, however, is still uncertain, since neither the smooth muscle, nor the striated muscle or myofibroblastic origin is sufficiently proven. Recent molecular studies revealed recurrent translocations affecting the chromosomes 12, 16 and 22 [4–6]. The resulting fusion genes involved in the pathogenesis of the lesion are FUS-ATF1, EWSR1-ATF1 (the latter being also the most frequent molecular alteration in clear cell sarcoma) and the most recently described EWSR1-CREB1 which turns out to be the most frequent rearrangement in AFH [7, 8].

AFH is usually characterized by rather bland histiocytoid proliferation. Greater degree of cellular pleomorphism, characteristically in the metastatic lesions, was described in the original paper in Cancer [1] . Pleomorphism in the

primary tumor was found to be occasional and focal, but rare diffuse pleomorphic cases causing differential diagnostic confusion are also known [9, 10]. The relationship of the pleomorphism to the genetic background is still uncertain.

Case Report

10-year-old boy with a 10 mm large subcutaneous lump on the right shoulder was admitted for surgery. He had no complaints at the physical examination and had no other disease. Local excision was made. At the gross inspection the removed lesion conveyed the impression of a lymph node and the sample was submitted for histology.

Materials and Methods

The specimen was bisected, formalin fixed, embedded in paraffin and 4 μ sections were cut and stained by hematoxylin and eosin according to standard histological protocol. Also, (3-Aminopropyl)-triethoxysilane-coated (Sigma) slides were prepared for immunohistochemical analysis. The primary antibodies (each from DAKO), the dilutions, pretreatments and incubation times used for the reactions are summarized in the Table 1. The developing system used for the reactions was the Envision from DAKO. Fluorescence in situ hybridization (FISH) for EWSR1/CREB1 fusion gene was performed according to the method published earlier with some modifications. The CTA-984G1 BAC probe covering the 5′ part of the EWSR1 locus was labeled with biotin-dUTP. The two fosmid clones G248P81788B12 and G248P89268A6 probes specific to the distal region of the CREB locus at 2q33.3 were labeled with Digoxigenin-dUTP. The hybridized probes were visualized by using an indirect detection scheme: the biotin labeled probes were incubated with Cy3-streptavidin conjugates followed by biotin labeled goat anti-streptavidin antibody and a repeated Cy3-streptavidin incubation. For the detection of Digoxigenin incubation with FITC conjugated mouse monoclonal antibody raised against Digoxigenin was followed by incubation with FITC-conjugated polyclonal Rabbit anti mouse antibody. Between the incubation procedures three changed washes were performed to remove the excess of non-bound conjugates. All incubation times and other washing conditions were identical as described earlier by Rossi et al. [8].

Results

Histological analysis of the specimen revealed a pleomorphic cell population showing epithelioid morphology. The tumor cells were arranged in compact sheets forming the center of the lesion which was surrounded by a dense lymphoid cuff mimicking the structure of a lymph node. At the periphery a fibrous capsule was identified. (Fig. 1a-b.) There was no prominent angiomatoid change or hemorrhage within the lesion and no mitoses or necrosis were found. The tumor cells showed diffuse, strong vimentin, focal and strong desmin, and moderate, also focal smooth muscle actin and EMA reaction, but all the other markers (S100, HMB45, CD1a, CD68, CD31, CD23, CD21, CD35 and AE1-AE3 cytokeratin) listed in the Table 1. were negative. (Fig. 1c.) FISH revealed co-localization of CREB1 (green) and EWSR1 (red) signals resulting in yellow color in some of the cells. In the pleomorphic nuclei of the tumor cells multiplication of the fusion signals was also seen (Fig. 1d.). A similar multiplication of centromere region specific alpha satellite probes were observed (data not shown) indicative of endomitosis resulting in multiplication of the whole genome.

Table 1 Summary of primary antibodies used for the study

Antibody/clone	Pretreatment	Dilution	Incubation
Vimentin/V9	Citrate/pH 6, 10 min.	1:100	60 min.
Desmin/D33	Citrate/pH 6, 10 min.	1:100	60 min.
SMA/1A4	Citrate/pH 6, 10 min.	1:100	60 min.
EMA/E29	Citrate/pH 6, 10 min.	1:100	60 min.
CD23/MHM6	TRS/pH 6, 20 min.	1:23	60 min.
CD21/1 F8	TRS/pH 6, 20 min.	1:20	60 min.
CD35/Ber-MAC-DRC	TRS/pH 6, 20 min.	1:25	60 min.
S100/polyclonal rabbit	Citrate/pH 6, 10 min.	1:2000	60 min.
Melanosome/HMB45	Citrate/pH 6, 10 min.	1:250	60 min.
CD1a/10	TRS/pH 6, 20 min.	1:25	60 min.
CD68/Kp1	Citrate/pH 6, 10 min.	1:1000	60 min.
CD31/JC70A	Citrate/pH 6, 10 min.	1:30	60 min.
CK/AE1-AE3	Citrate/pH 6, 20 min.	1:75	60 min.

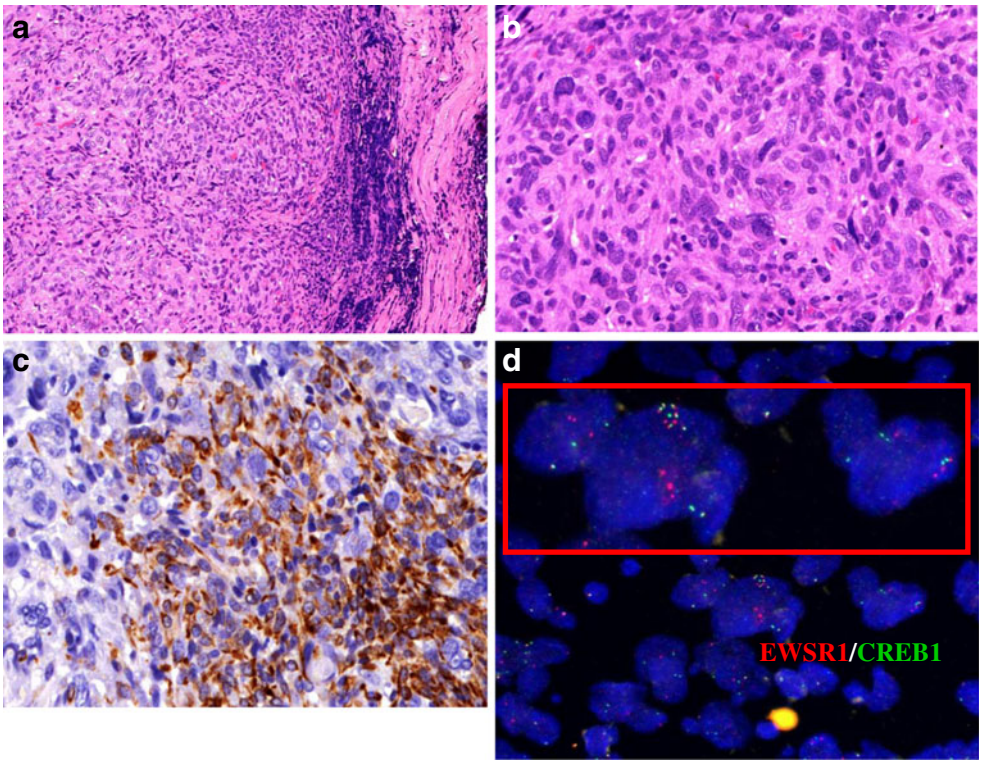

Fig. 1 At low power view the lesion shows a characteristic peripheral fibrous capsule and lymphoid cuff around the tumor tissue (**a**). At higher magnification the striking pleomorphism (**b**.) and the desmin reaction are visible (**c**). FISH indicates co-localization and multiplication of the EWSR1-CREB1 fusion gene (*red*/*green* signals). Insert shows the higher magnification (**d**)

Discussion

AFH is mainly a childhood neoplasm; however it may occur in any age group. It forms a well circumscribed subcutaneous nodule on the extremities, head and neck and trunk. The tumor characteristically has a cystic-hemorrhagic angiomatoid center and a dense peripheral lymphoid cuff mimicking the gross appearance of a lymph node. Microscopically it is a bland histiocytoid or fibroblast-like cell proliferation which shows few mitotic figures and is surrounded by lymphoid tissue. [1]

The case reported here showed some unusual features. There was no prominent angiomatoid change or hemorrhage within the lesion. Also, the cell population exhibited marked pleomorphism which, according to the literature, is characteristic in metastatic lesions rather than the primary tumor [1, 11]. The pleomorphic appearance raises the differential diagnosis of other pleomorphic tumors, sarcomas or metastases which are much more aggressive and have poorer prognosis [11]. Other rare phenotypic variants such as the recently described reticular-myxoid type may also cause differential diagnostic problems indicating the significance of using ancillary molecular techniques in these cases [12].

Few studies deal with the clinical aspects of the disease. In a larger study reviewing 108 cases, 12% local recurrence rate was identified but only one developed presumed distant metastases [9]. The results of the same study indicated that deep fascial invasion was the only predictor for metastasis. Pleomorphism and mitotic rate were not found to correlate with metastatic disease. In another study two of the ten examined patients developed metastases, where the tumor showed pleomorphism and high mitotic activity and the patients eventually died of the disease [11]. Our patient is well and without any evidence of the disease after more than 2-years follow-up. The last clinical and MRI examination found no local recurrence or distant metastases. The follow-up period is rather short to draw a firm conclusion, but the negative physical and radiological status seems to support the view that pleomorphism is not predictive for adverse outcome.

In the recent few years major advances have been made in determining the molecular genetic changes of the neoplasm. Three translocation variants with partly similar partners resulting in FUS-ATF1, EWSR1-ATF1 and EWSR1-CREB1 fusion genes were described [5–8, 10]. The most frequent translocation seems to be the EWSR1-CREB1, but the accumulating data indicate, that this is true only for the soft tissue cases [13]. In the non-soft tissue cases (primary bone, lung and cerebral tumors) the EWSR1-ATF1 fusion gene and its variants with the breakpoints at different exons is the basic change [13–15]. Looking at the data above the EWSR1-CREB1 fusion in our soft tissue case is not surprising, but multiplication or amplification of the fusion gene in the tumor cells has not yet been described. Presence of multiple copies of centromere region specific alpha satellite probes in conjunction with the fusion signals in the same tumor favors that the multiplication resulted from endomitotic reduplications and not the local amplification of

the translocated chromosomal regions. Inferring a relationship between the multiplication of chromosomal material including the fusion gene and the pleomorphic phenotype is logical, but their influence on prognosis is not clear. Based on the literature and follow-up of our case the pleomorphism alone without high mitotic rate probably does not indicate adverse outcome. The role of fusion gene multiplication, first described in the case reported here, needs to be studied further.

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Conflict of interest statement We declare that we have no conflict of interest.

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