PROFESSOR RAF SCIOT (Orcid ID : 0000-0003-2244-5839)

Article type : Correspondence

Primary myxoid mesenchymal tumor with intracranial location: report of a case with a *EWSR1*-*ATF1* fusion.

Corresponding author mail id : raf.sciot@uzleuven.be

About a third of soft tissue tumors are recognized by gene fusions. Some of these fusions are not histotype specific and occur in entities with totally different clinicopathological features. A good example is the fusion between *EWSR1* and genes of the *CREB* transcription factor gene family (*CREB1* or *ATF1*). These fusions are seen in angiomatoid fibrous histiocytoma, soft tissue and gastrointestinal clear cell sarcoma, primary pulmonary myxoid sarcoma and even in a non-mesenchymal tumor, the hyalinising clear cell carcinoma of salivary gland.[1]

Recently, eight unique myxoid mesenchymal tumors were described with fusions between *EWSR1* and *CREB1*, *ATF1*, or *CREM*, another gene of the *CREB* family. These tumors occurred in children and young adults and all but one were intracranial lesions. On histology, they were lobulated and myxoid, containing cords or strands of uniform round cells. Based on the unique clinicopathological features, a new entity was suggested, descriptively named as 'myxoid mesenchymal tumor with predilection for intracranial location.[2] In the latest report on three cases, the question was raised if this lesion could be related to the myxoid variant of angiomatoid fibrous histiocytoma.[3]

We recently encountered a lesion with identical clinicopathological features.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/his.13437

A 17 year old girl was hospitalized for the first time seven years ago for an increasing hemiparesis on the right side and an epileptic insult. On imaging, a cystic space occupying lesion of 5.9 cm diameter was seen in the frontal lobe, with minimal peri-lesional edema (Figure 1). A macroscopic complete resection was performed and the patient recovered from the paresis. Twenty months later, a recurrent lesion 2.5 cm was documented and incompletely resected, followed by radiotherapy (59.4 Gray). At that time the patient was free of symptoms. Because of slow progression of the residual nodule, a third resection was done recently, seven years after the initial diagnosis. Six months after this resection, the patient is free of disease.

On histology, all resected lesions showed a similar picture. A well demarcated, partly myxoid and lobular tumoral proliferation was seen. The cellularity was very variable, ranging from very myxoid to more compact (Figure 2A and 2B). The tumor cells were very monotonous and mainly showed a rounded aspect with clear to eosinophilic cytoplasm (Figure 2C and 2D). There was no nuclear atypia, and mitotic figures were not seen. Branching vessels were present, but there was no vascular proliferation. A fibrous pseudocapsule, peritumoral lympho-plasmacytic infiltrates, amianthoid-like fibers and blood-filled cystic spaces were lacking. Immunohistochemically, there was only expression of EMA (Figure 2E) and CD99. GFAP, IDH1 R132H, S100 protein, desmin, alfa-smooth muscle actin, NeuN, CD31, CD34, synaptophysin, chromogranin, and neurofilament stainings were negative. There was no loss of INI-1 or ATRX expression. On the first two resection specimens, no final diagnosis was put forward, just a descriptive conclusion of a benign or low grade partly myxoid lesion of unknown identity.

Cytogenetic analysis by G-banding using fresh tissue sample from the primary surgical specimen showed 45-46,XX,-13,t(12;22)(q13;q12)[cp20] karyotype (Figure 3A). Two separate break-apart FISH assays were performed on paraffin sections from primary tumor following standard procedure, using either dual-color *EWSR1* (Abbot Molecular, Des Plaines, IL) or custom-made *ATF1* (combination of BAC's RP11-189H16 and RP11-407N8 clones) break-apart probe set, as described previously.[2] By both

assays, approximately 80-85% of tumor cells showed one fused (red/green) signal and one green and red split signals per nucleus (Figure 3B), confirming the rearrangement of both, *EWSR1* and *ATF1* genes.

Frozen tumour tissue from a third resection was available for molecular karyotyping. DNA was extracted from the tumour specimen using High Pure PCR Template Preparation Kit (Roche). Array CGH (aCGH) analysis was carried out using the OGT CytoSure™ ISCA oligoarray set (Oxford Gene Technology, Oxford, UK), containing 180k DNA oligonucleotides with a minimum resolution of 200 kb. Microarray hybridization and copy number variant (CNV) analysis were performed according to the manufacturer's instructions. All genome coordinates were according to NCBI human genome build 37 (hg19, February 2009). aCGH analysis revealed only few copy number aberrations, as follows: arr[GRCh37] 2q24.1q31.1(156893603-170587701)x1, 6q22.31q25.1(125406258-151703036)x1, 13q12.12q12.13(23553332-26335543)x1, 13q12.2q34(27940855-115093155)x1 (Figure 3C). Of note, there was no overlap of these numerical changes with the genomic copy number alterations reported recently in the similar tumors by Bale *et al*.[3] Thus, these might be secondary genetic alterations of yet unknown biological significance.

Within the group of mesenchymal tumors with *EWSR1-CREB* gene family fusions, this newly described entity, of which we document the ninth case, shows histological overlap with the primary pulmonary myxoid sarcoma and myxoid angiomatoid fibrous histiocytoma. However, the first tumor presents as endobronchial lesions in adult patients and for the latter, a fibrous pseudocapsule, peritumoral lympho-plasmacytic infiltrates and blood-filled cystic spaces were lacking. There are two other myxoid mesenchymal tumors with *EWSR1* involvement, extraskeletal myxoid chondrosarcoma (EMC) and myoepithelial tumors. EMC typically occurs in the deep soft tissue of the limb in middle-aged adults and *NR4A3* is the translocation partner in the vast majority of cases. Myoepitheliomas also show histological overlap but combine expression of keratin and/or EMA with S100 protein and/or GFAP. Moreover, various translocation partners have been described, so far: *POU5F1*, *PBX1*,

PBX3, *ZNF444*, and *KLF17*. Only one myoepithelial tumor in the pelvis of an adult was reported to have a *EWSR1-ATF1* fusion, but the immunophenotype was typical for myoepithelioma.[1] Within the cranium, only three primary myoepitheliomas have been described, with typical expression of keratin, S100 protein and GFAP, but no documented *EWSR1* rearrangement.[4] Our case confirms the observation of recurrent genetic alteration involving *EWSR1* gene in myxoid mesenchymal intracranial tumors by Kao et al (2017) and Bale et al (2017), being the second case carrying *EWSR1-ATF1* fusion. Interestingly, amianthoid-like fibers are lacking in the two cases with *EWSR1-ATF1* fusion, but are reported in the six cases with the other fusions. The potential significance of this is not clear. A combined approach using immunohistochemistry and molecular analysis is recommended to separate this rare entity from its mimics. The potential relation with myxoid angiomatoid fibrous histiocytoma awaits further study.

Raf Sciot[1]

Sandra Jacobs[2]

Frank Van Calenbergh[3]

Philippe Demaerel[4]

Agnieszka Wozniak[5]

Maria Debiec-Rychter[6]

Department of Pathology[1], Pediatrics[2], Neurosurgery[3], Radiology[4], Oncology[5], and Human Genetics[6], KU Leuven and University Hospitals Leuven, Leuven, Belgium

References

1. Thway K, Fisher C. Tumors with EWSR1-CREB1 and EWSR1-ATF1 fusions: the current status. *Am. J. Surg. Pathol*. 2012; 36; e1-e11.
2. Kao YC, Sung YS, Zhang L at al. EWSR1 fusions with CREB family transcription factors define a novel myxoid mesenchymal tumor with predilection for intracranial location. *Am. J. Surg Pathol*. 2017; 41; 482-490.
3. Bale TA, Oviedo A, Kozakewich H at al. Intracranial myxoid mesenchymal tumors with *EWSR1-CREB* family gene fusions: myxoid variant of angiomatoid fibrous histiocytoma or novel entity? *Brain Pathol*. 2017; doi: 10.1111/bpa.12504 [Epub ahead of print].
4. Gupta K, Klimo P, Wright KD. A 2 year old girl with dysmetria and ataxia. *Brain Pathology* 2016; 26; 126-127.

Figures description

Figure 1. Axial FLAIR image shows a cystic lesion with surrounding vasogenic edema. On the sagittal T1 weighted image after intravenous administration of Gadolinium, an enhancing nodule can be seen in the upper wall of the cyst.

Figure 2. At low power H&E stain, a well delineated lobulated tumor is seen (A). Slightly higher power shows the transition from more solid to myxoid areas, as well as the branching vessels (B). The tumor cells are very monotonous (C). In the more solid areas, the tumor cells have the same appearance with a clear to eosinophilic cytoplasm (D). EMA expression is present (E).

Figure 3. Cytogenetic features of the tumor. G-banded karyotype from the primary lesion reveals translocation t(12;22)(q13;q12), as pointed by red arrows, and loss of chromosome 13 (A). Break-apart FISH assay shows *EWSR1* rearrangement in tumor cells, as evident by split red and green signals (B). Genomic profile of recurrent lesion by array-CGH discloses small deletions within chromosomes 2 and 6, and the whole chromosome 13 loss (C).