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Sclerosing Epithelioid Fibrosarcoma–A Report of Two Cases with Cytogenetic Analysis of *FUS* Gene Rearrangement by FISH Technique

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Abstract Sclerosing epithelioid fibrosarcoma (SEF) is a rare soft tissue sarcoma. Recently, a link has been suggested between SEF and low-grade fibromyxoid sarcoma (LGFMS) on the basis of the finding of the characteristic translocation t(7;16) (*FUS-CREB3L2*) of LGFMS in a small number of studied cases of SEF. The frequency of this translocation in SEF is still unknown. We present 2 cases of SEF with cytogenetic analysis for *FUS* rearrangement. The tumors occurred in 12 and 58 year old patients, respectively and consisted of a well to partially circumscribed, non-encapsulated mass, comprising monomorphic, polygonal cells arranged in aggregates, cords and single file arrays in a variably sclerotic stroma. The cells exhibited minimal nuclear atypia with moderate amount of clear to eosinophilic cytoplasm and rare mitotic figures. One case also showed bland spindle cell areas with myxoid change, as seen in LGFMS. By immunohistochemistry (IHC), the tumor cells were diffusely positive for vimentin, focally for S-100 in 1 case and negative for cytokeratin (CK), epithelial membrane antigen (EMA), HMB-45, desmin, smooth muscle actin (SMA), H-caldesmon, Myo D-1, CD34 and CD 168. By fluorescent in-situ hybridization (FISH) technique, the case with mixed SEF and LGFMS histology was positive for *FUS* rearrangement. Our study reinforces the previously reported relationship between SEF and LGFMS, and suggests that SEF may represent a variant of LGFMS in at least some cases, rather than an entirely distinct fibrosarcoma variant.

Keywords Sclerosing epithelioid fibrosarcoma · Low-grade fibromyxoid sarcoma · Soft tissue sarcomas · FISH technique · *FUS* rearrangement

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Introduction

Sclerosing epithelioid fibrosarcoma (SEF) is a relatively newly described malignant fibroblastic tumor, considered by the current WHO classification of soft tissue and bone tumors, to represent a distinct variant of fibrosarcoma [1]. SEF is generally considered a low-grade sarcoma, although a more aggressive clinical course has been suggested in one relatively large series [2]. As described, SEF occurs over a wide age range in patients of either sex as a deeply situated, often large soft tissue mass, typically in the lower extremities [1–6]. Although SEF has traditionally been considered a distinct entity, a recent report has documented the presence of the low-grade fibromyxoid sarcoma (LGFMS)-associated translocation t (7; 16) (q32-34; p11) (*FUS-CREB3L2*) in a small number of studied cases of SEF, suggesting a link between these two tumors [7]. We herein report 2 cases of SEF identified at Tata Memorial Hospital, Mumbai, India over a 5 year period, including cytogenetic analysis of *FUS* gene rearrangements.

Case Histories

Case 1

A 12 years old girl presented to Tata Memorial Hospital with swelling over her left thigh of 2 months duration, unassociated with pain or ulceration. On clinical examination, a non tender swelling was noted over her left thigh measuring 4×4 cms^2. There was no skin ulceration. A previous fine needle aspiration biopsy, elsewhere, had been reported as showing a "proliferative fibrohistiocytic tumor". Review of FNAC was reported a sarcoma. A core needle biopsy and subsequent wide excision were performed.

Case 2

A 58 years old gentleman presented to an outside hospital with a left thigh mass of unknown duration and size. Slides and paraffin blocks were received at Tata Memorial Hospital for pathologic review.

Material and Methods

Paraffin section immunohistochemistry (IHC) was performed at Tata Memorial Hospital, to vimentin (monoclonal, 1:50, Dako, Produkionsveg, Glostrup, Denmark), cytokeratin (CK) (monoclonal, 1:100, Dako), epithelial membrane antigen (EMA) (monoclonal, 1:100, Dako), desmin (monoclonal, 1:50, Dako), smooth muscle actin (SMA) (monoclonal, 1:20, Dako), Myo D-1 (monoclonal, 1:20, Dako), Myogenin (1:50, Novocastra, UK), S-100 (polyclonal, 1:300, enzymatic antigen retrieval, Dako), HMB-45 (monoclonal, 1:50), p63 (monoclonal, 1:50, Dako), CD34 (monoclonal, 1:100, Dako), CD68 (monoclonal, 1:50, Dako), CD 163 (monoclonal, 1:100, Diagnostic Biosystems, USA), H-caldesmon (monoclonal, 1: 100, Dako) and MIB-1 (monoclonal 1:50, Dako), using a polymer based detection system (Biocare Med. MACH2 Universal polymer detection), and 3-3' diaminobenzidine trihydrochloride (DAB) chromogen.

Fluorescent in-situ hybridization for *FUS* break-apart analysis was performed at Mayo Clinic, Rochester, MN, USA using a *FUS* break apart probe (Vysis, Des Plaines, IL) according to a previously reported protocol [8]. Tumor samples were considered positive if more than 10% of 100 cells analyzed showed rearrangement. Tumors were evaluated and scored by 2 independent investigators.

Histopathological Findings

For case1, the gross specimen consisted of a soft tissue mass measuring 7.5×6×4 cms, with overlying skin. On cut section, a 3.5×3×2 cm tumor was well circumscribed within the surrounding muscle, firm, white and homogenous-appearing. The surgical margins were grossly negative for tumor (Fig. 1a). Microscopically, the morphologic features of the 2 tumors were very similar, consisting of a circumscribed, but infiltrative proliferation of relatively monomorphic epithelioid cells with lightly eosinophilic to clear cytoplasm and moderately hyperchromatic nuclei, indistinct nucleoli along with focal intranuclear pseudoinclusions, embedded in a densely collagenized to hyalinized fibroblastic matrix (Fig. 1b, c). Mitotic activity was very low and necrosis was absent. Cord-like, nested and single file growth patterns were present. Scattered foci of metaplastic bone formation were present in first case. (Figure 1d) The tumor from first case also showed small areas of more spindled growth, focally showing an "abrupt" transition to myxoid nodules with a whorling growth pattern and curvilinear arcades of small blood vessels, identical to the typical histological features of LGFMS (Fig. 1e, f). Masson's Trichome and Van Gieson stains highlighted abundant collagen in case 1.Reticulin stain highlighted areas of increased reticulin fibers, surrounding individual cells. By IHC, both cases were diffusely vimentin positive while focally, weakly S100 protein positive in one case. Desmin, H-caldesmon, SMA, CK, EMA, CD 34, HMB45, p63 and CD163 were negative. MIB-1 proliferative index was low.

By FISH, case 1 was positive for a *FUS* rearrangement in 60% cells, whereas the second case was negative. The dual colored 'break-apart' signals indicating *FUS* rearrangement and non-separate signals have been displayed. (Figure 2).

Discussion

Sclerosing epithelioid fibrosarcoma (SEF), first described by Meis-Kindblom et al. [3] in 1995, is a rare fibroblastic soft tissue tumor, currently considered by the WHO to represent a distinct variant of fibrosarcoma [1]. As described by Meis-Kindblom and colleagues [3] in their seminal series of 25 cases, SEF consists of monomorphic rounded to epithelioid cells arranged in cords and nests in a densely sclerotic stroma, with ultrastructural fibroblastic differentiation. Although this initial report concentrated on the epithelioid cytomorphology of the neoplastic cells, these authors also noted the presence in the majority of cases of areas resembling conventional fibrosarcoma, as well myxoid zones, reminiscent of myxofibrosarcoma or low-grade fibromyxoid sarcoma (LGFMS) [1, 3]. Interestingly, this initial report also noted expression of epithelial markers in a significant subset of cases, with EMA and CK expression in 50% and 14% of studied cases, respectively

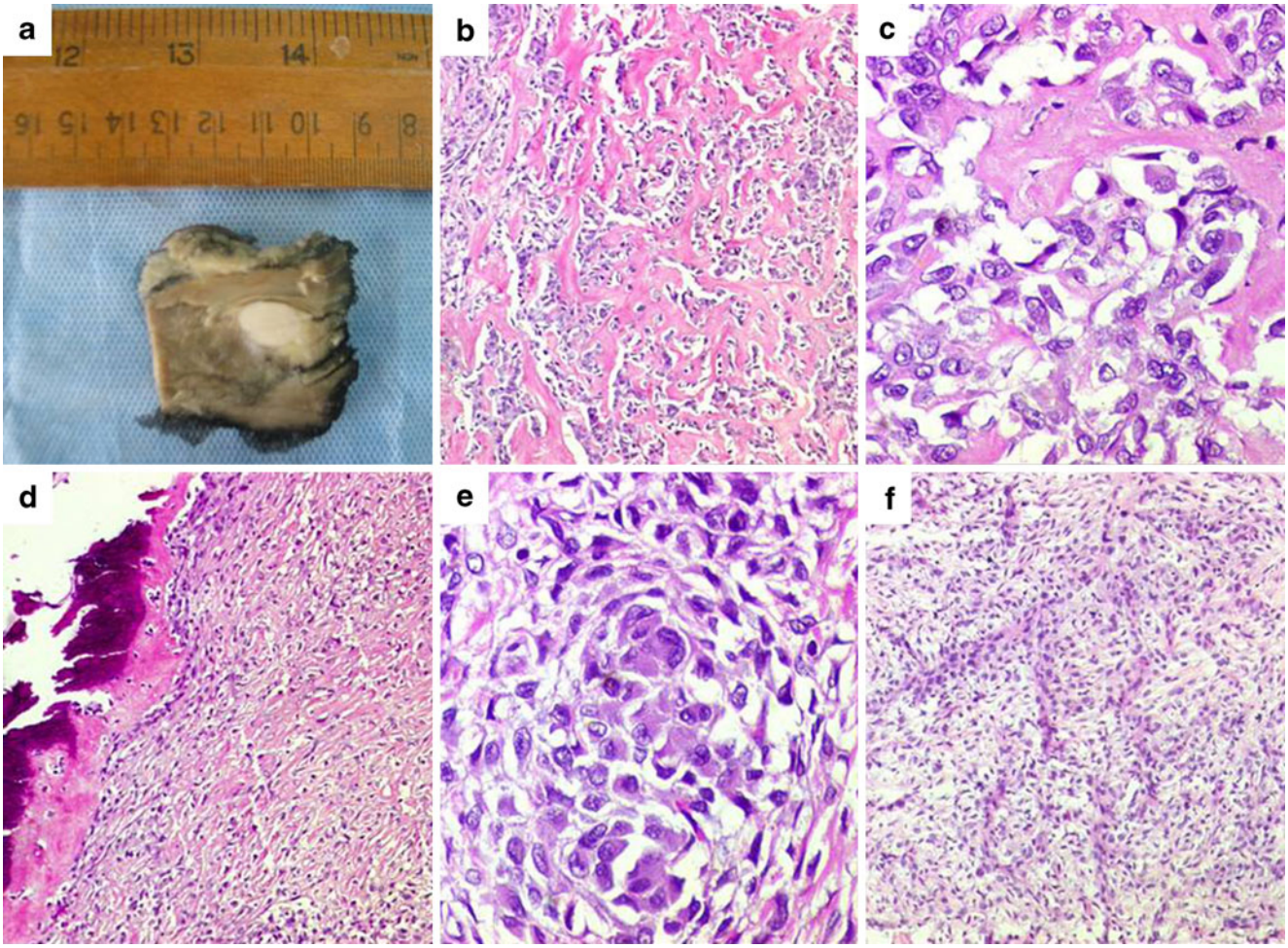

Fig. 1 Sclerosing epithelioid fibrosarcoma. **a** Gross features. A well circumscribed, homogenous pearly-white tumor surrounded by muscles and free inked margin. **b** Microscopic features. **b** Tumor exhibiting 'filigree' like areas of abundant eosinophilic, collagen deposition (sclerosis) H&E × 100. **c** Tumor cells with polygonal/epithelioid shape and moderate amount of clear to eosinophilic to clear cytoplasm separated by dense sclerotic stroma. H&E × 200. **d** Focal metaplastic bone formation within tumor. **h** & **e** × 100. E. Arcades of blood vessels akin to LGFMS in case 1. **f** Early rosettes of cells with moderate amount of eosinophilic cytoplasm and vesicular, bland nuclei. H&E × 200

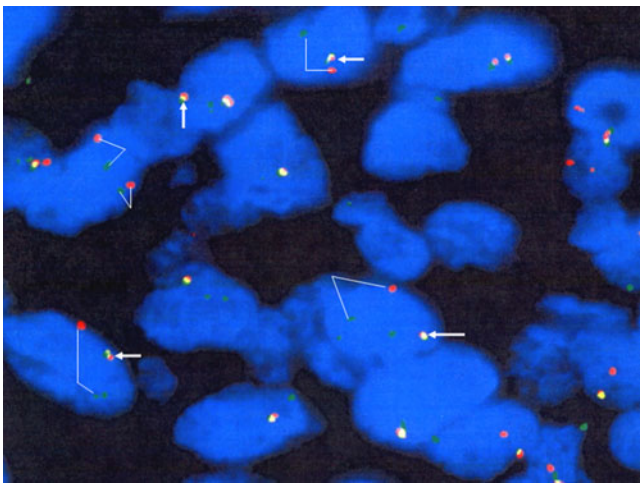

Fig. 2 Fluorescent in situ hybridization results .Case 1 showing dual color 'break-apart' of *FUS* gene probe resulting in 1 red and 1 green signal (*double lines*) in many cells. The 'non-separate' signals have been marked with thick arrows. DAPI × 1,000

[3]. Although Meis-Kindblom and co-workers [3] considered SEF to represent a relatively low-grade fibrosarcoma, local recurrences and distant metastases were noted in 53% and 43% of patients with follow-up, respectively.

The possibility that SEF might not represent a single histopathological entity was first raised by Eyden and colleagues [4] in a morphologic, immunohistochemical and ultrastructural study of 5 SEFs. Noting the high incidence of epithelial marker expression in the earlier study of Meis-Kindblom et al [3], and the possibility that such cases might represent entities other than "fibrosarcoma", Eyden and colleagues [4] proposed a strict definition of SEF requiring a "vimentin only" immunophenotype and ultrastructual demonstration of a pure fibroblastic phenotype, in the form of abundant rough endoplasmic reticulum and a Golgi apparatus producing collagen secretory granules.

A possible link between SEF and low-grade fibromyxoid sarcoma (LGFMS) was first explicitly suggested by

Antonescu and colleagues [2], who noted the presence of hypocellular collagenous zones and juxtaposed myxoid nodules, identical to LGFMS, in 4 of 16 studied SEF. Although Antonescu et al. [2] did not specifically hew to the criteria for SEF proposed by Eyden et al [4], their cases all showed ultrastructural fibroblastic differentiation, lacked CK expression, and showed only limited, weak EMA expression in a minority of cases. Putting together their data with that of Meis-Kindblom et al. [1, 3] and Eyden et al. [4], Antonescu and co-workers [2] concluded that SEF was an aggressive sarcoma, with an overall recurrence rate of >50%, a metastatic rate of 43–86%, and a tumor-specific mortality of 25–57%.

Additional evidence linking SEF and LGFMS comes from two genetic studies of LGFMS, from Guillou et al. [7] and Reid et al. [9]. Guillou and colleagues [7] from the French Sarcoma Group studied 63 tumors with morphological features of LGFMS and 66 no-LGFMS for the LGFMS-specific translocation t(7;16)(q32-34;p11)(*FUS-CREB3L2/L1*). Positive results were found in 81% of putative LGFMS and only 7 of 52 (13%) controls; of these 7 positive non-LGFMS, 4 had been previously labeled "SEF". Along similar lines, 1 of 4 genetically confirmed LGFMS reported by Reid et al. [9] showed at least in part classical features of SEF.

Our findings of areas histologically identical to LGFMS and *FUS* gene rearrangement in 1 of 2 studied SEF reinforce these prior studies, and provide additional evidence supporting a possibly close relationship between SEF and LGFMS, in at least a subset of cases. Additionally, it is known that areas identical to SEF may be seen in a variety of other soft tissue and bone tumors, including ossifying fibromyxoid tumor of soft parts, rare sclerosing Ewing sarcoma/ primitive neuroectodermal tumors, and sclerosing rhabdomyosarcoma among others [10–12]. Thus, one might ask whether SEF truly exists as a discrete entity, or instead represents a distinctive form of tumor progression, most often in LGFMS, but occasionally in other tumors as well. As noted above, the possibility that "SEF" may not represent a discrete diagnostic entity in some of the earlier diagnosed cases has been previously suggested by Eyden and colleagues[4]. Heterogeneity of cases previously reported as "SEF" may also account for the differences in prognosis noted in prior studies [1–4]. Obviously, study of a larger number of well-characterized SEF with modern immunohistochemical and molecular genetic methods will be necessary to address these important questions.

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