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Ossifying Fibromyxoid Tumor of Soft Parts: A Clinicopathologic, Proteomic and Genomic Study

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Abstract

Ossifying fibromyxoid tumor of soft parts (OFMT) is a rare soft tissue and bone tumor of borderline malignancy displaying an uncertain line of differentiation. The existence of fully malignant OFMT is controversial. In order to better understand the natural history and line of differentiation taken by OFMT, we studied 46 cases by light microscopic, immunohistochemical (IHC), genomic, proteomic, and fluorescent in situ hybridization (FISH) methods. Cases were classified according to the 2003 Folpe and Weiss system. Clinical and follow-up information was obtained. IHC for S-100 protein, desmin, epithelial membrane antigen (EMA), cytokeratins, smooth muscle actin (SMA), INI-1, neurofilament protein (NFP), CD56 excitatory amino acid transporter-4 (EAAT4), and MUC4 was performed on formalin-fixed, paraffin-embedded (FFPE) tissues. Gene expression profiling and proteomic studies were performed on FFPE tissues from 13 and 5 cases, respectively. FISH for *INI-1* was performed on 10 cases. The 46 tumors arose in 29 males and 17 females (median age 52 years, range 39-63 years) and involved the proximal (N=17) and distal extremities (N=13), head and neck (N=9) and trunk (N=5). Median tumor size was 5.4 cm (range 1.0-21.0 cm). Cases were classified as typical OFMT (26 of 46, 57%), atypical OFMT (5 of 46, 11%) and malignant OFMT (15 of 46 cases, 32%). Clinical follow-up (27 cases, median 55 months duration) showed all patients with typical and atypical OFMT to be alive without disease. Adverse events, including 3 local recurrences, 3 metastases, and 3 deaths, were seen only in malignant OFMT. IHC results were: S100 protein (30/41, 73%), desmin (15/39, 38%), cytokeratin (4/35 11%), EMA (5/32, 16%), SMA (2/34, 6%), INI-1 (lost in mosaic pattern in 14/19, 74%), EAAT4 (31/39, 80%), MUC4 (3/14, 21%), NFP (8/10, 80%) and CD56 (6/14, 43%). Gene expression profiling showed typical and malignant OFMT to cluster together, distinct from schwannian tumors. Proteomic study showed expression of various collagens, S100 protein, and neuron-related proteins. FISH showed *INI-1* deletion in 5/7 (71%) cases. We conclude that malignant OFMT exist, and may be recognized by the previously proposed criteria of Folpe and Weiss. Expression of neuron-related, in addition to Schwann cell and cartilage-associated markers,

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suggests a “scrambled” phenotype in OFMT. Loss of *INI-1* or other genes on 22q are likely important in the pathogenesis of these rare tumors.

Keywords

ossifying fibromyxoid tumor of soft parts; immunohistochemistry; gene expression profiling; proteomics; fluorescence in situ hybridization; *INI-1*

Introduction

Ossifying fibromyxoid tumor of soft parts (OFMT), first described by Enzinger et al in 1989 (10), is a rare soft tissue and bone tumor displaying an uncertain line of differentiation. In its classical form, OFMT is characterized by subcutaneous location, a peripheral shell of woven bone, and a vaguely lobular proliferation of small, bland round cells with very low mitotic activity, embedded in a fibromyxoid matrix (10). Although most OFMT conform to this morphologic description and show correspondingly benign clinical behavior, it has been recognized for some time that a subset of OFMT display atypical cytoarchitectural features, such as high cellularity or elevated mitotic activity, and show correspondingly more aggressive clinical behavior (13, 18, 39). Under the risk stratification system proposed in 2003 by Folpe and Weiss, OFMT are classified as “typical”, “atypical” or “malignant” based on their size, nuclear grade, cellularity, and mitotic activity (13). However, this risk stratification system is not universally accepted, with some authors for example suggesting that morphologically and clinically malignant OFMT do not exist, representing instead other tumor types (21).

The precise line of differentiation taken by OFMT has also remained controversial. Enzinger et al, in their original description, noted OFMT to have ultrastructural features suggestive of incomplete schwannian and/or cartilaginous differentiation (10). Subsequent immunohistochemical studies have also for the most part supported the concept of schwannian differentiation in OFMT (13, 16, 20, 29, 30, 39), although myoepithelial differentiation has also been suggested (18, 22).

Given these uncertainties, further study of these rare soft tissue tumors is clearly in order. We therefore undertook a comprehensive study of a large series of well-characterized OFMT, retrieved from the institutional and consultation files of Mayo Clinic, with the goals of: 1) further elucidating the natural history of OFMT, 2) reassessing the validity of the Folpe and Weiss classification scheme for OFMT, 3) clarifying the line of differentiation taken by OFMT through comprehensive immunohistochemical study, gene expression profiling and proteomic study, and 4) identifying potential new markers of this rare tumor.

Materials and Methods

Approval for this study was granted by the Institutional Review Boards of the participating institutions. All available slides for 54 Mayo Clinic internal and consultation cases previously diagnosed as “OFMT” for the period 1998-2010 were retrieved and re-reviewed by two of the authors (RPDG and ALF). Following re-review, 8 cases were felt to represent something other than OFMT and were excluded, leaving a final study population of 46 cases. Cases previously diagnosed as “malignant” OFMT were included only if clear areas of morphologically “typical” OFMT were present. S100 protein expression by the tumor was not required for inclusion in the final study population. Demographic and clinical follow-up information was obtained from Mayo Clinic medical records and from contributing pathologists and clinicians. All cases were re-classified as “typical”, “atypical”

and “malignant” according to the previously published criteria of Folpe and Weiss (13). Briefly, this classification system defines typical OFMT as those showing low nuclear grade and low cellularity and mitotic rate $< 2/50$ high powered fields (HPF). In contrast, malignant OFMT show high nuclear grade or high cellularity and mitotic activity $> 2/50$ HPF. Atypical OFMT deviate from typical OFMT but fail to meet criteria for malignancy. Tumors showing foci of nuclear crowding with loss of intervening matrix, occupying at least one 4x microscopic field were considered to show high cellularity.

Immunohistochemistry (IHC) for all markers except MUC4 was performed at Mayo Clinic, using 4-micron thick formalin-fixed, paraffin embedded (FFPE) tissue sections and commercially available antibodies directed against S-100 protein (polyclonal, 1:50-1:100, Dako Corp, Carpinteria, CA, USA), desmin (DE-R-11, 1:50-1:100, Novocastra, Newcastle upon Tyne, UK), epithelial membrane antigen (EMA) (E29, 1:50-1:100, Dako Corp.), cytokeratins (OSCAR, 1: 40, Signet, Dedham, MA, USA) smooth muscle actin (SMA) (1A4, 1:100, Dako Corp.), and INI-1 (clone 25, 1:40, BD Transduction Laboratories, Franklin Lakes, NJ, USA). Following our DNA microarray results suggesting expression of neuron-related genes in OFMT (see Results, below) subsets of cases were evaluated for expression of neurofilament protein (2F11, 1:100, Dako Corp.), CD56 (123C3, 1:25, Dako Corp.), and excitatory amino acid transporter-4 (EAAT4), a protein normally expressed in cerebellar Purkinje cells (goat polyclonal, 1:100, Santa Cruz, Santa Cruz, CA). Steam heat induced epitope retrieval and the Dako Envision detection system (Dako Corp., Carpinteria, CA) were utilized. MUC4 IHC was performed at the University of Nebraska, using previously published methods (32). Appropriate positive and negative controls were employed. The immunohistochemical results were scored as “negative” (no positive cells), “rare” ($< 5\%$ of cells positive), “1+” (5–25% cells positive), “2+” (26–50% positive), and “3+” ($> 50\%$ positive).

Gene microarray study was performed at UCLA, on FFPE blocks from 13 OFMT (8 typical and 5 malignant). Tumor was macrodissected and microarray performed using previously published and validated methods (31). Total RNA was isolated using the Ambion RecoverAll (Applied Biosystems/Ambion, Austin, TX, USA) kit according to the manufacturer’s instructions. RNA integrity was evaluated using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) and purity/concentration was determined using a NanoDrop 8000 (NanoDrop Products, Wilmington, DE, USA). Microarray targets were prepared using NuGEN WT-Ovation Formalin-Fixed Paraffin-Embedded RNA Amplification System and FL-Ovation cDNA Biotin Module V2 (NuGEN Technologies, San Carlos, CA, USA) and then hybridized to the Affymetrix GeneChip U133 Plus 2.0 Array (Affymetrix), all according to manufacturers’ instructions. The arrays were washed and stained with streptavidin phycoerythrin in Affymetrix Fluidics Station 450 using the Affymetrix GeneChip protocol, and then scanned using an Affymetrix GeneChip Scanner 3000. The acquisition and initial quantification of array images were conducted using the AGCC software (Affymetrix). The subsequent data analyses were performed using Partek Genomics Suite Version 6.4 (Partek, St Louis, MO, USA). Cluster analysis and principal component analysis were conducted with Partek default settings. Biofunctional analysis was performed using Ingenuity Pathways Analysis Software Version 7.6 (Ingenuity Systems, Redwood City, CA, USA). The results were compared to previously obtained data from nerve sheath myxomas and schwannoma (31). Thereafter, typical and malignant OFMT were compared to each other. Differentially expressed genes were selected at ≥ 5 -fold and $P < 0.005$.

Proteomic studies were performed at Mayo Clinic. For proteomic studies, areas of tumor were microdissected from FFPE sections using laser microdissection microscopy followed by trypsin digestion, nano-flow liquid chromatography, electrospray ionization and tandem

mass spectrometry (MS/MS) as previously described (27, 35). The MS/MS data were correlated with known theoretical fragmentation patterns of tryptic peptide sequences from the Swissprot database using the Scaffold algorithm (35).

Molecular cytogenetic studies with fluorescence in situ hybridization (FISH) were performed on 10 cases of typical OFMT at St. Jude Children's Research Hospital on 4 µm formalin-fixed, paraffin-embedded tissue sections using a laboratory-developed dual-color probe containing *INII* [CTD-2511E13 + CTD-2034E7] (22q11.2) (Orange fluorochrome) probe as the target probe and *PANX2* [RPCI3-402G11] (22q13.33) (Green fluorochrome) probe as the control. The paraffin-embedded tissue was pretreated in a 60°C oven for 30 minutes, deparaffinized in xylene/isopropanol, and pressure cooked in a citrate buffer for 60 minutes. The tissue was digested by placing the slide in a coplin jar containing 4 mg/ml of pepsin at 37°C for 3-5 minutes, depending on the tissue density. The probes were codenatured with the target DNA at 90°C for 12 minutes on a ThermoBrite slide processing system and hybridized at 37°C for 24 hours. Following hybridization, slides were washed and counterstained with DAPI- Vectashield mounting solution (Vector). Hybridization signals were analyzed with an Olympus epifluorescence microscope equipped with Applied Imaging software. By in-house validation studies, cut-off values of 41%, 23%, and 9% were established for loss of one copy of 22q, hemizygous deletion of *SMARCB1* (*INII*), and homozygous deletion of *SMARCB1* (*INII*), respectively.

Results

Clinical and Microscopic Features, including Clinical Follow-up

The 46 tumors arose in 29 males and 17 females, with a median age of 52 years (range 39-63 years). The tumors arose predominantly in the soft tissues of the proximal (N=17) and distal extremities (N=13), but also involved the head and neck (N=9) and the trunk (N=5). The median tumor size was 5.4 cm (range 1.0-21.0 cm). Radiographically, the tumors appeared as non-specific soft tissue masses, on occasion showing surrounding or intralesional calcification on plain radiographs or CT scans. Grossly, the tumors were generally well-circumscribed, occasionally had a visible shell of bone, and showed a vaguely lobulated appearance with a gritty cut surface.

By definition, all tumors showed, at least in part, classical morphological features of OFMT as previously described (10, 13) (Figure 1). A partial shell of woven bone was present in 29 of 48 (60%) cases including 19 of 26 (73%) typical, 3 of 5 (60%) atypical and 7 of 15 (47%) malignant OFMT. Briefly, typical OFMT (26 of 46 cases, 57%) were composed of a low to at most moderately cellular proliferation of very bland, uniform, round to slightly ovoid cells embedded in a myxoid, fibromyxoid, or hyalinized stroma. Mitotic activity was <2/50 high powered fields (HPF); necrosis and vascular space invasion were absent. In addition to areas of typical OFMT, malignant OFMT (15 of 46 cases, 32%) contained areas showing some combination of high nuclear grade, high cellularity, and mitotic activity >2/50HPF (13). Necrosis and infiltrative growth were seen in subsets of malignant cases. Histologically malignant foci could be identified at the time of initial presentation (13 of 15 cases, 87%) (Figure 2) or in subsequent local recurrences of initially typical OFMT (2 of 15 cases, 13%) (Figure 3). Five of 46 cases (11%) were classified as atypical OFMT.

The clinical follow-up data is summarized in Table 1. Clinical follow-up was available for 27 cases (59%), with a median of 55 months duration (range 12-149 months). All patients with typical OFMT were alive without disease at the time of last clinical follow-up. Similarly, both patients with atypical OFMT with follow-up were disease free at the time of last clinical contact. In contrast, adverse events were seen in 33% of patients with malignant OFMT with follow-up, including 2 patients with local recurrences (3 events), 3 patients with

distant metastases, and 3 deaths from disease. One patient with a malignant OFMT is known to be alive with persistent disease.

Immunohistochemical Results

The immunohistochemical results are summarized in Table 2. In agreement with previous studies, expression of S100 protein was seen in the majority of studied cases (30 of 41 cases, 73%), including 88% of typical, 75% of atypical and 42% of malignant OFMT. S100 protein expression was typical present at 1-2+, and was seldom diffusely positive (Figure 4A). Malignant OFMT typically showed greater S100 protein expression in typical, as compared to malignant-appearing areas. Anomalous desmin expression was also relatively common, present in 15 of 39 (38%) of cases, including 43% of typical, 50% of atypical and 25% of malignant tumors (Figure 4B). Cytokeratin, epithelial membrane antigen and smooth muscle actin expression were seen in only small numbers of cases, typically at 1+. Loss of INI-1 expression in 30-60% of lesional cells ("mosaic pattern") was noted in 14 of 19 (74%) tested cases (Figure 4C).

Following identification of *EAAT4* and *MUC4* overexpression by DNA microarray (see Gene Expression Profiling Results, below) subsets of OFMT were tested for *EAAT4* and *MUC4* expression. Patchy (1-2+) *EAAT4* expression was noted in 31 of 39 (80%) tested cases (Figure 4D). *MUC4* was positive in 3 of 14 (21%) tested cases (Figure 4E). Focal (1+) expression of other neuron-associated markers, neurofilament protein and CD56, was present in 80% and 43% of cases, respectively (Figures 4F and G).

Gene Expression Profiling Results

The gene expression profiling results, comparing typical OFMT to schwannoma and nerve sheath myxoma, are illustrated in Venn diagram (Figure 5A), cluster map (Figure 5B) and principal component analysis map (Figure 5C). As shown in the Venn diagram, OFMT show >1000 differentially expressed genes as compared with nerve sheath myxomas and schwannomas, tumors which have been previously shown to have similar expression profiles (31). The differences between OFMT and nerve sheath myxoma/schwannoma are further illustrated in the cluster and principal component analyses, showing OFMT to clearly segregate from the latter two entities. Selected genes up- and down-regulated in OFMT as compared with nerve sheath myxoma/schwannoma are listed in Table 3. Of the up-regulated genes, *EAAT4* is normally expressed principally by neuronal cells, whereas the remainder of this group does not show tissue restriction. Figure 5D illustrates relative *EAAT4* expression levels in OFMT as compared with schwannoma and nerve sheath myxoma. The 2 down-regulated genes listed, peripheral myelin protein 22 (*PMP22*) and myelin expression factor 2 (*MYEF2*) are both typically expressed in Schwann cells. Figure 5E illustrates relative *PMP22* expression levels in OFMT as compared with schwannoma and nerve sheath myxoma. A very large number (>1000) of other genes, without known tissue restriction, were also down-regulated in OFMT as compared with schwannian tumors (data not shown). Importantly, no significant differences in gene expression profiles were noted between typical and malignant OFMT, supporting the relationship of these two groups of tumors.

Proteomic Results

Mass spectrometry based proteomic analysis identified a total of 896 unique proteins in the five tumor samples studied (Figure 6). These included identified a large number of matrix proteins in OFMT, including collagens types 1A1, 1A2 and 6A3. Type 2 collagen (COL2A1), associated with cartilage and cartilage-producing tumors, was present in 2 of 5 cases studied. Other proteins identified in abundance included members of the family of S-100 proteins, versican (a neuron-associated glycoprotein), katanin (a neuron-associated microtubule severing protein) and numerous histones.

FISH Results

All cases studied with FISH were known to show “mosaic” pattern *INI-1* expression by IHC. Seven of the 10 tested cases had a successful hybridization; in 2 the FISH test showed a normal signal pattern distribution and the other 5 had an abnormal signal pattern (Table 4). These five cases had hemizygous deletion of *INI-1* and *PANX2* (the control probe) in a significant population of cells (43% to 89%, mean, 65%) (Figure 7A). Of interest, in 3 of these cases, an additional abnormal population was identified, showing 2 signals for *INI-1* and 1 signal for the control, consistent with the loss of the telomeric region of 22q in one chromosome without *INI-1* deletion (Figure 7B).

Discussion

The present study was designed to address a number of questions about OFMT, the first (and perhaps most important) of which is: “Do malignant OFMT exist, and if so, can we reliably recognize them?” We believe our study to answer both of these questions in the affirmative. In our opinion, histologically malignant OFMT clearly exist. Such tumors are defined by the presence of clear-cut areas of morphologically typical OFMT juxtaposed to areas showing high nuclear grade, high cellularity and elevated mitotic activity, while maintaining the overall cytoarchitectural features of OFMT (e.g., lobularity, fibromyxoid matrix, uniform cell-cell spacing and non-pleomorphic round to ovoid cells. Indeed, we have yet to see a completely convincing example of histologically malignant OFMT in which areas of ordinary OFMT were not present, either in the primary tumor or (even more convincingly) in an earlier presentation.

The relationship between typical and malignant OFMT is also supported by our gene expression profiling and immunohistochemical data. We observed very similar expression profiling patterns in both typical and malignant OFMT when compared to nerve sheath myxoma/schwannoma (e.g., upregulation of *EAAT4* and *MUC4* and downregulation of *PMP22* and *MYEF2*), as well as no significant differences when typical and malignant OFMT were directly compared. Immunohistochemically, typical and malignant OFMT showed roughly similar frequency of expression of the various markers tested, albeit with diminished expression of S100 protein in malignant OFMT, and an increased frequency of focal, weak expression of epithelial markers (e.g. cytokeratins and EMA). Diminished S100 protein expression in malignant OFMT likely reflects malignant progression in the neoplastic cells, akin to the diminished CD34 expression that often accompanies fibrosarcomatous transformation in dermatofibrosarcoma protuberans (1). In particular, we believe that our finding of a “mosaic” pattern of *INI-1* protein loss in identical percentages of typical and malignant OFMT (discussed further, below) argues persuasively in favor of their relationship, as this is a highly unusual pattern of *INI-1* protein expression, previously reported only in rare schwannomas associated with familial schwannomatosis (26) and in some synovial sarcomas (19). Obviously, different results would be expected if malignant OFMT represented instead other sarcoma types. Given our results, we believe the prior suggestion by Miettinen and colleagues that histologically and clinically malignant OFMT do not exist reflects their arbitrary definition of OFMT as a tumor without atypical features (21).

Our data support the validity of the risk stratification system for OFMT proposed initially by Folpe and Weiss in 2003 (13), inasmuch as we observed clinically malignant behavior (e.g., aggressive local recurrences and distant metastases) only in tumors fulfilling criteria for malignant OFMT. The relatively high percentage of malignant OFMT (32%) in the present study likely reflects the unique nature of our consultation practice. We did not observe any metastases in cases classified as typical or atypical OFMT, although metastases have been previously documented in 4% and 6% of cases falling into those categories, respectively

(13). Metastases were not observed in typical OFMT studied by Miettinen et al, although local recurrences were present in 22% of patients with follow-up (21). Clinically malignant behavior in OFMT has also been documented in several other small studies, typically in cases showing histologic features suggestive of malignancy (18, 24, 28, 37-39). Putting together the results of the present study with those of earlier studies, it is likely that the metastatic risk of typical OFMT is less than 5%, supporting the current WHO classification of OFMT as an “intermediate (rarely metastasizing)” mesenchymal tumor (12). Cases fulfilling criteria for malignant OFMT should, however, be regarded as high-grade sarcomas.

Turning to the question of the line of differentiation taken by OFMT, our data suggest that OFMT show a “scrambled” phenotype, with limited expression of schwannian and cartilaginous markers, as previously noted, as well as expression of a variety of neural markers, a novel finding. Although schwannian differentiation has generally been favored in OFMT, based on ultrastructural findings, such as the presence of well-developed, occasionally reduplicated external lamina, and S100 protein expression (8, 11, 16, 20), our gene expression data does not support this. Both by cluster analysis and principal component analysis OFMT were clearly distinct from nerve sheath myxoma and schwannoma, both highly differentiated schwannian neoplasms. Expression of some Schwann cell-related genes, such as *PMP22* and *MYEF2*, was in fact down-regulated in OFMT as compared with nerve sheath myxoma/schwannoma. Cartilaginous differentiation has also been suggested in OFMT, based on ultrastructural features such as irregular cell borders with short processes and intracellular microfilaments, and S100 protein expression (10). Although we were not able to compare OFMT to cartilaginous tumors by gene expression profiling, we did identify collagen II production in 2 of 5 cases studied by proteomic methods, suggesting that at least some OFMT may show limited cartilaginous differentiation. S100 protein expression in OFMT, shown in this study both by proteomic study and by immunohistochemistry, might also suggest some element of schwannian or cartilaginous differentiation in these tumors, although a wide variety of non-schwannian/ non-cartilaginous cell types may of course express S100 protein (36). Finally, our findings of only very infrequent cytokeratin or smooth muscle actin expression would seem to offer little support for the notion of myoepithelial differentiation in OFMT.

Surprisingly, we found OFMT to express some neuron-associated genes and proteins, by gene expression profiling, proteomic study and immunohistochemistry. DNA microarray identified a neuron-associated gene, *EAAT4*, to be expressed at high levels in OFMT as compared with schwannian tumors. *EAAT4* is a member of the high affinity glutamic acid and neutral amino acid transporter family, expressed principally in the cerebellar cortex (7, 14). We were able to confirm *EAAT4* protein expression in 80% of OFMT by immunohistochemistry. Our microarray data also identified another neuron-related gene, *HuC*, as being over-expressed at a 4-5 fold level, with a highly significant p-value (data not shown). However, we were not able to identify a *HuC* antibody applicable to FFPE tissues, and were thus unable to validate this finding. We did, however, find limited expression of the well-established neuronal markers neurofilament protein and CD56 (neural cell adhesion molecule) in 75% and 41% of tested OFMT, respectively. Finally, proteomic study identified abundant katanin, a neuron-associated microtubule severing protein (2) and versican, a neuron-associated proteoglycan (3) in tested cases of OFMT. Although it is obviously premature to ascribe neuronal differentiation to OFMT, in the absence of any convincing light microscopic or ultrastructural supporting evidence, these findings are intriguing. Conceivably OFMT may be showing limited, aberrant expression of neuronal markers due to yet to be defined, dysregulating, upstream genetic events. The significance of expression of *MUC4*, a mucin-related protein expressed by various epithelia, in OFMT is obscure.

INI-1 is a putative tumor suppressor gene located on chromosome 22q11.2 which encodes a protein which is expressed essentially in all nucleated cells. Homogeneous loss of immunoreactivity has been identified in most cases of epithelioid sarcoma, (6, 17, 25) atypical teratoid rhabdoid tumor, (4, 5) rhabdoid tumor of the kidney and malignant extrarenal rhabdoid tumor (33), with mutations of *INI-1* thought to play a pathogenetic role in tumorigenesis (23, 34). As noted above, we observed an unusual loss of *INI-1* protein expression in 30-60% of neoplastic cells ("mosaic pattern"), a finding previously reported only in schwannomas associated with familial schwannomatosis and in some synovial sarcomas (19, 26). Our FISH studies for *INI-1* suggest that 22q may carry genes with some possible pathogenic relevance in this tumor. Most examined cases showed aneuploidy for chromosome 22 in a significant population of cells suggesting a possible pathogenic role for *INI-1* in OFMT. While none of the 7 cases we evaluated were found to have a homozygous deletion for *INI-1*, 5 cases showed a hemizygous deletion of both *INI-1* and *PANX2* (the control probe) on average in more than 50% of cells, and 3 of the 5 had a second population of cells showing 2 signals for *INI-1* and 1 signal for the control, suggesting loss of one copy of the 22q telomeric region. However, the molecular events leading to the loss of expression in a subpopulation of tumor cells are unclear. In the heterozygous state for the gene deletion, we hypothesize that epigenetic events such as post translational modifications, or genetic aberrations that are not detectable by FISH such as small deletions or mutations could contribute to the loss of function of the *INI-1* on the intact chromosome. On the other hand, the finding of a second existing population of cells with the loss of distal 22q without *INI-1* loss raises the possibility that other genes in this chromosomal region, such as the *NF2* gene, implicated in neurofibromatosis type II (15), may play a crucial role in the pathogenesis of OFMT. In any event, we have been able to confirm aneuploidy for the long arm of chromosome 22 in a subset of tested cases, strongly suggesting some role for the *INI-1* or an adjacent locus in the pathogenesis of OFMT. Interestingly, mosaic pattern loss of *INI-1* was seen both in morphologically and clinically typical OFMT, as well as in malignant OFMT. No difference in behavior was noted in tumors showing retained *INI-1* expression versus those with mosaic pattern *INI-1* loss. We are not aware of any reports of OFMT arising in the setting of familial *INI-1* or *NF2* gene mutations, nor were such histories present in any of our cases.

In conclusion, we believe the results of our study amply confirm the existence of malignant OFMT, as well as the validity of the current risk stratification system for OFMT. Although the line of differentiation taken by this rare tumor remains enigmatic, expression of neuron-related markers in OFMT appears to be significantly more common than is expression of schwannian or cartilaginous markers. The significance of expression of neuronal markers in OFMT remains to be fully elucidated. *EAAT4* may prove to be a novel diagnostic marker of use in the differential diagnosis of OFMT, although study of large numbers of potential OFMT mimics is required before these can be recommended. *MUC4* is unlikely to be a useful marker of OFMT, particularly as it is known that *MUC4* is frequently expressed in at least one potential mimic of OFMT, low-grade fibromyxoid sarcoma (9). The role of *INI-1* loss and 22q aneuploidy in OFMT also warrants further study.

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Figure 1.
“Typical” ossifying fibromyxoid tumor, showing a peripheral shell of woven bone (A), low to intermediate cellularity (B), and bland, round to ovoid cells with uniform cell-cell spacing, embedded in a fibrous (C) to myxoid (D) matrix.

Figure 2.

Malignant ossifying fibromyxoid tumor, “de novo” type. This tumor arose in the groin of a middle aged male, and showed a shell of woven bone on CT scan (A). At scanning magnification, areas of hypocellular typical ossifying fibromyxoid tumor are juxtaposed to areas showing greater cellularity and nuclear enlargement (B). At higher power magnification, the malignant areas of this tumor showed high nuclear grade and mitotic activity. Note shell of woven bone (C). This patient suffered multiple pleural and pulmonary metastases and died of disease (D).

Figure 3.

Malignant ossifying fibromyxoid tumor arising in typical ossifying fibromyxoid tumor. At the time of initial presentation, this patient had a typical ossifying fibromyxoid tumor, showing low to intermediate cellularity and low nuclear grade (A). Four years later, the local recurrence of this tumor showed high cellularity (B), high nuclear grade, and elevated mitotic activity (C). A second local recurrence, one year later, also showed vascular space invasion (D). This patient eventually died of disease.

Figure 4. Representative images of immunohistochemical results in ossifying fibromyxoid tumors. Variable S100 protein expression (A). Anomalous desmin expression (B). “Mosaic” pattern loss of INI-1 expression (C). Expression of EAAT4 (D) and MUC4 (E), validating gene expression profiling results. Focal expression of neurofilament protein (F) and CD56 (G).

Figure 5. Venn diagram (A), cluster map (B) and principal component analysis map (C) showing similar expression profiles in tested cases of ossifying fibromyxoid tumor, distinct from nerve sheath myxoma and schwannoma. Overexpression of EAAT4 (D) and under expression of PMP22 (E) were seen in ossifying fibromyxoid tumors.

Figure 6.
Selected proteins identified at high levels by mass spectrometric analysis in ossifying fibromyxoid tumors.

Figure 7.

Fluorescence in situ hybridization, showing only one signal for *INI-1* and for *PAX2* (control), consistent with monosomy 22/or loss of one 22q, in a significant population of cells (A). In 3 cases, an additional abnormal population was identified, showing 2 signals for *INI-1* and 1 signal for the control, suggesting loss of the 22q telomeric region in one chromosome without *INI-1* deletion (B).

Table 1

Clinical Follow-Up

	Follow-up (Number, median, range)	Local Recurrences	Metastases	Alive without disease	Alive with disease	Dead of Disease
Typical (N=26)	17, 57 mos, (12-149 mos)	0	0	17 (100%)	0	0
Atypical (N=5)	2, 39.5 mos, (24-55 mos)	0	0	2 (100%)	0	0
Malignant (N=15)	9, 28 mos, (17-139 mos)	2 patients, 3 events	3 patients	5 (56%)	1 (11%)	3 (33%); all with metastases
All cases (N=46)	28, 55 mos (12-149 mos)	2 patients, 3 events	3 patients	24 (86%)	1 (4%)	3 (10%); all with metastases

Table 2

Immunohistochemical Results

	S100 protein (%)	Desmin (%)	CK (%)	SMA (%)	EMA (%)	NF (%)	CD56 (%)	EAAT4 (%)	MUC4 (%)	INI-1 (%)
Typical OFMT	22/25 (88)	10/23 (43)	1/20 (5)	0/21 (0)	0/20 (0)	8/10 (80)	6/14 (43)	18/25 (72)	1/6 (16)	Mosaic: 8/12 (75); retained: 4/12 (25)
Atypical OFMT	3/4 (75)	2/4 (50)	0/4 (0)	0/3 (0)	0/3 (0)	1/1 (100)	0/1 (0)	3/4 (75)	0/2 (0)	Mosaic: 3/3 (100)
Malignant OFMT	5/12 (42)	3/12 (25)	3/11 (27)	2/10 (20)	5/9 (56)	3/5 (60)	1/2 (50)	10/10 (100)	2/6 (33)	Mosaic: 3/4 (75); retained 1/4 (25)
Overall	30/41 (73) (1+; 12; 2+; 16; 3+; 2)	15/39 (38) (1+; 8; 2+; 7)	4/35 (11) (1+; 3; 2+; 1)	2/34 (6) (1+; 2)	5/32 (16) (1+; 2; 2+; 3)	12/16 (75) (1+; 12)	7/17 (41) (1+; 7)	31/39 (79)	3/14 (21)	Mosaic: 14/19 (74); retained: 5/19 (26)

Table 3

Selected Genes Differentially Expressed by Ossifying Fibromyxoid Tumor, Nerve Sheath Myxoma and Schwannoma

Gene Symbol	Gene Title	P-value(OFMT vs. NSM)	Fold-Change(OFMT vs. NSM)	P-value(OFMT vs. Schwannoma)	Fold-Change(OFMT vs. Schwannoma)
DLK1	delta-like 1 homolog (Drosophila) solute carrier family 1 (high affinity aspartate/glutamate transporter), member	0.0005	11.399	0.0003	10.9952
EAAAT4/SLC1A6	member HERV-H LTR-	<0.0005	10.683	<0.0005	7.73536
HHLA2	associating 2 Mucin 4, cell	<0.0005	6.24505	<0.0005	5.08082
MUC4	surface associated peripheral myelin	0.003	5.47792	0.003	5.24875
PMP22	protein 22 myelin expression	<0.0005	-40.026	<0.0005	-72.8112
MYEF2	factor 2	0.0005	-6.09082	0.0003	-6.2514

OFMT: ossifying fibromyxoid tumor; NSM: nerve sheath myxoma

Table 4

Detailed findings in 5 OFMT cases with aneuploid pattern for 22q by FISH, targeting *INI-1* and *PANX2*

Case #	Monosomy 22/loss of 22q (%)	Hemizygous deletion of <i>PANX2</i> (control gene) alone (%)	Hemizygous deletion of <i>SMARCB1 (INI1)</i> alone (%)	Normal pattern (%)
1	89	<1	<1	9
2	73	<1	<1	25
3	64.5	16.5	<1	18
4	56	39	<1	4
5	43	52	<1	4