Bone & Soft Tissue

18 Osteosarcoma of the Hands and Feet: A Clinico-Pathologic Distinct Subgroup of OS?

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Background: Osteosarcoma (OS) of hands or feet represent less than 1% of all cases. From the sparse case reports in literature it seems that patients with these locations differ in clinical course, radiologic and/or pathological presentation, compared to the usual location of OS.

Design: In order to evaluate these individual observations, we studied a large cohort patients with OS in hand or foot, obtained from the merged Dutch Nation-wide Automated Pathological Archive (PALGA) and the Archives of the Netherlands Committee for Bone Tumours (NCBT) and that of the Istituto Ortopedica Rizzoli (IOR), Bologna, Italy, respectively. Clinical details, radiology and pathology reports were reviewed in most cases. The results of the patients were compared with published cases.

Results: Thirty-nine cases with OS localized in the hands (n=12) or foot (n=27) were identified in the data bases (1.7% of all OS). In concordance with 54 well documented individual case reports in literature of Hand OS another 54 such cases of Foot OS showed that patients in our data set were in the 4^{th}-5^{th} decade at diagnosis, older than usual. Male female ratio in these series was 2.0:1 vs 1.65:1 in the literature for OS of the foot, but OS of the hands thr M:F ratio was 1:1, different from the literature (M:F=1.38:1). The patient history was around 1year in this series, and pain and swelling were the most likely presenting symptom of OS in both hands and feet. In our series, more OS were located in the metacarpals (58%) than in the phalanges (25%) of the hands, similar as in the literature. In the foot, the tarsal and metatarsal bones were in 56% and 33% involved, which was more or less like was found in the literature. The phalanges were affected in 11%. Histologically, 30% of OS in this series were low grade (nearly all LG central OS), whereas in the literature 20% (foot) - 24%(hand) were low grade OS. Although the most frequent subtype was the (high-grade) osteoblastic variant, but 8%-15% of the high grade OS were of the unconventional variants. Using standard OS-chemotherapy protocols for high grade OS in combination with adequate surgery, overall survival in the our series was > 70%.

Conclusions: OS in hand and feet is rare and represent < 1% of all OS, have different and peculiar clinical and histological features but should be approached similar like high grade OS in more common sites.

19 Frequent PLAG1 Gene Rearrangements in Skin and Soft Tissue Myoepithelioma (ME) with Ductal Differentiation

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Background: A subset of cutaneous and superficial ME tumors display a distinct ductal component and closely resemble mixed tumors/pleomorphic adenomas of salivary gland. As *PLAG1* and *HMGA2* gene rearrangements are the most common genetic events in pleomorphic adenomas, we sought to investigate if these abnormalities are also present in the skin/soft tissue ME lesions. In contrast, half of the deep-seated soft tissue ME tumors lacking ductal differentiation are known to be genetically unrelated, showing *EWSR1* gene rearrangements.

Design: FISH analysis to detect *PLAG1* and *HMGA2* gene abnormalities was performed in 33 ME tumors, 8 from skin and 25 from soft tissue, lacking *EWSR1* gene rearrangements. For the *PLAG1* rearranged tumors, FISH and RACE was performed to identify potential fusion partners, including *CTNNB1 (beta-catenin)* on 3p21 and *LIFR (leukemia inhibitory factor receptor)* on 5p13.

Results: Recurrent *PLAG1* gene rearrangement by FISH was detected in 11 (33%) lesions, including 2 (25%) in the skin and 9 (36%) in the soft tissue. All were classified as benign morphologically and all except one showed abundant tubulo-ductal differentiation (comprising 10/22 [45%] of all tumors with ductal structures). A *PLAG1-LIFR* fusion was detected by RACE and then confirmed by FISH in one soft tissue ME tumor with tubular formation. No *CTNNB1* or *LIFR* abnormalities were detected in any of the remaining *PLAG1*-rearranged tumors. No *HMGA2* gene abnormalities were detected in any of the 22 ME lesions tested.

Conclusions: A subset of cutaneous and soft tissue ME tumors appear genetically linked to their salivary gland counterparts, displaying frequent PLAG1 gene rearrangements and occasionally *PLAG1-LIFR* fusion.

20 Angiomatoid Fibrous Histiocytoma: An Expansion of the Histologic Spectrum

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Background: Angiomatoid fibrous histiocytoma (AFH) is a tumor of intermediate malignancy that usually presents on extremities of children/young adults. Classically, AFH is composed of histiocyte-like cells with round to oval nuclei with pseudovascular spaces, hemorrhage and hemosiderin and is surrounded by a fibrous pseudocapsule with a peripheral lymphocytic cuff. AFH frequently have translocations involving *EWSR1*, or less commonly *FUS*. Morphologic variants have been described, but some cases of AFH cause diagnostic difficulty and this tumor is a frequent source of consultation. We reviewed our experience with AFH and emphasize unusual histologic features.

Design: Detailed histologic examination was performed, evaluating classic features, unusual features and mitotic rates. In cases with blank slides/blocks, immunostains for CD68, desmin and EMA and FISH for the *EWSR1* translocation were performed. Demographics were also reviewed.

Results: 26 cases met selection criteria. A broad morphologic spectrum was observed. In addition to classic histologic features, 9 cases displayed various degrees of sclerosis with 3 displaying a focal swirling perineurioma-like pattern, with 1 having a diffuse perineurioma-like pattern. Nine cases had at least moderate pleomorphism, with 2 having striking pleomorphism. 8 had eosinophils, one with numerous eosinophils throughout the tumor. Atypical mitotic figures were seen in 3, including two pleomorphic AFHs and the case with numerous eosinophils. One had reticular/myxoid morphology. Evidence of a *EWSR1* translocation was seen in 11/15 tested. One pleomorphic AFH was aneusomic for *EWSR1*, suggesting a cryptic translocation. Immunohistochemically, 69% expressed EMA, 53% desmin and 63% CD68. With the exception of one case, all had ≥2 of the classic findings: peripheral lymphocytic cuff, fibrous pseudocapsule, pseudovascular spaces, hemorrhage and hemosiderin. The single exception only had a lymphocytic cuff. In review of the demographics data, 5 (19%) occurred in patients >40 years old, and 9 (35%) were located outside the extremities. Three (12%) occurred in patients >40 years old and in atypical locations.

Conclusions: AFH has a broader histologic and clinical spectrum than is commonly recognized. In addition to conventional features, sclerosis, perineurioma-like areas, marked eosinophilia, atypical mitotic figures, marked pleomorphism, and complex cytogenetic aberrations involving *EWSR1* may be seen. Many typical histologic features may be absent. Knowledge of this morphologic spectrum with utilization of FISH is helpful in AFH with unusual features.

21 Liposarcoma of the Mediastinum and Thorax- 22 Cases in an Uncommon Location with Diverse and Unusual Histology

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Background: Liposarcoma (LPS) rarely occurs in the mediastinum and thorax. Recent delineation of the genetic underpinnings of LPS has led to classification into three principal subtypes: well differentiated LPS/dedifferentiated LPS (WDL/DL), myxoid LPS (ML) and pleomorphic LPS (PL). Most previous reports of mediastinal LPS predate this genetically based classification. Herein we report the clinicopathologic and molecular genetic features of a series of thoracic LPS identified over a 60 year period.

Design: The consultation archives of the authors and surgical pathology database of our institution were searched for cases coded as lipoma and LPS of the mediastinum and thorax. The slides were reviewed, yielding 22 confirmed cases of LPS. Cases were reclassified using the most recent WHO classification. Follow up information was obtained from medical records and referring clinicians. Florescence *in situ* hybridization (FISH) for *CPM* amplification and/or *DDIT3 (CHOP)* rearrangement was performed on selected cases.

Results: The 22 cases occurred in 12 men and 10 women (mean 52 years, range 15-73). 21 cases arose primarily in the mediastinum (6 anterior, 6 posterior, 2 middle, 2 superior, 5 multiple compartments), and 1 arose in the inferior pleural space. The tumors were typically large (mean 15.7 cm, range 8-27 cm). All subtypes were encountered with 8 WDL, 5 DL (2 confirmed *CPM*+), 4 ML, 4 PL (one confirmed *CPM*-) and 1 unclassifiable liposarcoma (*CPM*- and *DDIT3*-). Unusual histologic features were noted in many cases, including extensively myxoid WDL mimicking ML (2 cases), WDL with low-grade smooth muscle differentiation (lipoleiomyosarcoma, 1 case), DL with osteosarcomatous and "meningothelial"-like dedifferentiation, extensively differentiated ML mimicking WDL (*CPM*-), and PL with epithelioid and myxoid change. Clinical follow up was available on all patients with a post-resection interval >12 months (mean follow up 60 months, range 12-252). Patient outcome varied significantly with histologic subtype, with death from disease occurring in 1 of 6 WDL, 1 of 4 DL, 3 of 4 ML and 4 of 4 PL.

Conclusions: Although all LPS subtypes may occur in the mediastinum, this location seems to show an unusual preponderance of uncommon subtypes (e.g., PL) as well as unusual morphological variants of common subtypes (e.g., myxoid WDL). Correct classification of mediastinal LPS has important clinical implications, with most patients with WDL/DL having a protracted clinical course, in contrast to the more rapid disease progression seen in patients with ML and PL.

22 Gene Deletion Underlies Loss of P16 Expression in Osteosarcoma Tumors with Poor Response to Neoadjuvant Chemotherapy

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Background: Although pathologic response to neoadjuvant chemotherapy predicts survival among patients with osteosarcoma (OS), there are currently no established molecular markers to predict response to chemotherapy. We have previously shown that immunohistochemical (IHC) expression of p16 (the product of the CDKNA2 gene) in OS is significantly correlated with pathologic response to neoadjuvant chemotherapy. The objective of the current study was to assess for copy number alternations at the CDKNA2 gene on chromosome 9 in p16 IHC-expressing and IHC-non-expressing OS specimens.

Design: Genomic DNA was obtained from paraffin-embedded pretreatment biopsy specimens of OS patients prior to receiving neoadjuvant chemotherapy. We selected three cases of p16 IHC-expressing tumors with pathologic response to chemotherapy (≥ 90% tumor necrosis) and three cases of P16 IHC-non-expressing tumors without pathologic response (< 90% tumor necrosis). Human genomic array was performed on 44K arrays using 80 Gb per human genome at 30X coverage. The CDKN2A locus (p16) on chromosome 9 was probed with A_14_P129522 chr9, A_14_P130650 chr9 and A_14_P112983 chr9.

Results: Four of the 6 cases yielded complete copy number information (2 p16 IHC-expressing, 2 p16 IHC-non-expressing). Among p16 expressing tumors, both demonstrated a wildtype CDKNA2 locus, while one of two p16 non-expressing tumors had a deletion on the short arm of chromosome 9 including the CDKNA2 locus.

Conclusions: Deletion of the entire CDKNA2 locus explains loss of p16 expression in some OS patients with poor response to neoadjuvant chemotherapy. However diverse mechanisms may be responsible for loss of p16 expression across the population of OS patients. Additional molecular studies are needed to validate these findings.

23 Is Routine Histopathologic Examination of Femoral Heads Justified? A 10-Year Review of Clinicopathologic Discrepancies in Elective Hip Arthroplasty Specimens at Two Institutions

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Background: The utility of routine histopathologic examination of hip arthroplasty specimens has long been a source of controversy between orthopedic surgeons and surgical pathologists. Absolute consensus does not exist as to the appropriate handling of these specimens. We examined the discrepancy rate between clinical and histopathologic diagnosis in hip arthroplasty specimens at two medical institutions to determine whether microscopic examination of femoral heads is indeed justified.

Design: A 10 year retrospective review of 8921 hip arthroplasty specimens was performed at two medical institutions. Clinical diagnosis was compared with final pathologic diagnosis for each case, and major and minor discrepancies were documented.

Results: Discrepancy rates for the individual institutions were 12.3% and 18.8%. Overall, there was a combined discrepancy rate of 18.1% between the submitted clinical and final pathologic diagnosis in a total of 1614 patients. 4.9% of all cases exhibited major discrepancies, in patients for whom the treatment and/or prognosis was altered following histopathologic diagnosis. The remaining discrepancies were considered minor, with more academic than clinical/therapeutic significance (e.g. osteoarthritis versus avascular necrosis of the femoral head). Major discrepancies included both clinically unsuspected histopathologic findings and suspected processes not confirmed by histopathologic examination. Table 1 lists major discrepancies.

Table 1. Major clinicopathologic discrepancies

Diagnosis	Clinically unsuspected	Clinically suspected but not identified histopathologically
Malignancy	28	4
Pigmented villonodular synovitis	19	1
Enchondroma	27	0
Bone island	67	0
Degenerative joint disease in hip dysplasia	291	50
Osteomyelitis	2	1

Conclusions: Clinical and histopathologic diagnoses of hip arthroplasty specimens were compared at two separate institutions, yielding a relatively high overall discrepancy rate of 18.1%. 4.9% of all cases harbored major discrepancies, which included but were not limited to: 32 malignancies, 20 cases of pigmented villonodular synovitis, and 3 cases of osteomyelitis. These major diagnostic discrepancies significantly altered management and/or long-term prognosis. Consequently, histopathologic examination of all hip arthroplasty specimens is, in our opinion, highly justified.

24 Morphologic and Immunophenotypic Analysis of Desmoid-Type Fibromatosis Following Radiation Therapy

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Background: The morphologic changes seen in desmoid-type fibromatosis following ionizing radiation have not been well studied, and the range of cytologic atypia, cellularity, mitotic activity, and lesional necrosis for irradiation effect in desmoid vs. post-radiation sarcoma has not been assessed.

Design: The histopathologic and immunophenotypic features of a series of primary and locally recurrent desmoid fibromatoses resected at variable intervals after radiation therapy (XRT) were compared with paired pathologic specimens obtained prior to XRT. Of 190 cases of desmoid tumor in the surgical pathology archives, only 23 patients had been treated with ionizing radiation and only 8 specimens had been resected following XRT.

Results: Excluding two cases resected less than 35 days after completion of XRT, the median interval between XRT and surgical resection was 108.7 months (range, 4.5-191 months). All patients were treated with 180-200 cGy fractions to a dose of between 50-60 Gy. Histological and immunophenotypic characteristics of desmoid-type fibromatoses resected before and after XRT were not significantly different in 7 of the 8 cases studied. Logistic regression analysis disclosed no significant associations between the post-XRT interval and histologic characteristics or staining patterns of myogenic markers. The most common histologic alterations (seen in only 3 cases) were subtle vascular changes. One locally recurrent desmoid tumor resected 191 months after XRT showed morphologic evidence of fibrosarcomatous transformation, with zonal necrosis, hypercellularity, severe nuclear atypia, and increased mitotic activity.

Conclusions: In rare cases of recurrent desmoid tumor, the differential diagnosis may include radiation-induced fibrosarcoma. Our data suggest that histomorphologic alterations in desmoids attributable to the effects of ionizing radiation are minimal. Therefore, the presence of cytologic and architectural features of malignancy in this setting warrants careful examination and consideration of the rare occurrence of post-radiation sarcoma. Lesser degrees of nuclear atypia seen in isolation do not necessarily indicate a poor prognosis in irradiated desmoid-type fibromatosis.

25 Primary Fibrosarcoma of Bone (PFSB): A Re-Evaluation of Cases Seen at a Single Institution for the Period 1913 – 2009

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Background: PFSB, currently defined by the WHO as a "primary malignant spindle cell neoplasm of bone in which the tumor cells are typically organized in a fascicular or 'herringbone' pattern", is thought to account for <5% of primary bone tumors. However, its true incidence is difficult to discern, owing to changes in diagnostic terminology over time and improved recognition of fibrosarcoma (FS) mimics with modern ancillary techniques. We re-evaluated a large series of tumors previously diagnosed as FS involving bone in order to more precisely define criteria for the diagnosis of PFSB in the modern era, and to better elucidate the incidence of such tumors.

Design: All available slides from 161 cases diagnosed as PFSB involving bone for the period 1913 -2009 were retrieved from our archives. Clinical records, pathology and radiology reports were reviewed. Secondary FSs, cases without adequate slides, and FSs secondarily involving bone were excluded, leaving a study population of 97 cases. Criteria for diagnosis included: 1) spindle cell morphology and a fascicular growth pattern, 2) no more than moderate pleomorphism, 3) absent osteoid production, and 4) absent expression by immunohistochemistry (IHC) of other than limited smooth muscle actin (SMA). IHC for cytokeratins (OSCAR and AE1/AE3), SMA, desmin, S-100, and CD34 was performed using commercial antibodies and the Dako Envision detection system. IHC for TLE-1 failed due to specimen decalcification.

Results: Of the remaining 97 study cases, 64 were considered not to meet morphological criteria for PFSB. These were predominately reclassified as pleomorphic sarcomas. This left a study group of 33 cases. Of these, 15 were reclassified as leiomyosarcoma, 7 as probable synovial sarcoma, and 1 as myoepithelioma. 10 cases met our criteria for PFSB; these occurred in 6 F and 4 M (age range 6 to 76 yrs) and involved the femur (3), sacrum (2), and 1 each in the fibula, humerus, tibia, pubis, and scapula.

Conclusions: Using strict diagnostic criteria and ancillary IHC, we found PFSB to be extremely rare, accounting for <1% of primary bone sarcomas seen at our institution. PFSB should be distinguished from its many potential mimics, most commonly leiomyosarcoma and synovial sarcoma. The natural history of PFSB remains to be fully established, particularly as prior series of such tumors have likely included what we would now consider to represent non-PFSB. On-going molecular genetic studies should help to more clearly establish the incidence of reclassified osseous synovial sarcoma.

26 Pathologic Response to Neoadjuvant Radiotherapy (NRT) as Potential Prognostic Factors in Soft Tissue Sarcomas (STS)

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Background: This study aims to elucidate the prognostic value of pathologic response to NRT in STS and its correlation to RECIST criteria and survival outcomes (SO).

Design: 84 patients aged 19-92 years (mean, 57 years) who underwent resection for STS after NRT were analyzed, including 36 patients who also received neoadjuvant chemotherapy (NCT). Pathologic response was assessed by scoring % of viable tumor, necrosis, fibrosis, and other stromal changes. Other pathologic (tumor size, histologic type, tumor grade, margin status) and clinical parameters (age, sex, location, radiologic response by changes in maximal tumor diameter and RECIST criteria, and SO) were obtained.

Results: Mean % of viable tumor, necrosis, fibrosis, and other stromal changes was 30.8%, 22.2%, 41.2%, and 5.8%, respectively. Pre-NRT and post-NRT MRI results available in 50 patients showed median % change in maximal tumor diameter of 1.4% (range, -60.2 to +55.7%). By RECIST criteria: 8 patients had progressive disease, 37 had stable disease, and 5 had partial response. Tumors that decreased in maximal diameter post-RT exhibited less necrosis ($16 \pm 4\%$ vs. $36 \pm 7\%$, $p=0.015$) and more fibrosis ($53 \pm 5\%$ vs. $23 \pm 6\%$, $p=0.001$) than tumors that increased in maximal diameter. Comparable findings were found when pathologic response was analyzed with respect to RECIST criteria. Tumors with >80% fibrosis were associated with better SO than tumors with moderate (10-80%) or minimal fibrosis (<10%) ($p=0.003$, overall survival). Tumors with >5% necrosis were associated with worse SO. Intermediate-grade tumors exhibited higher degree of viable tumor ($45 \pm 8\%$ vs. $27 \pm 3\%$, $p=0.014$) and lower degree of necrosis ($4 \pm 1\%$ vs. $27 \pm 3\%$, $p=0.001$) compared to high-grade tumors. Tumors <10cm showed higher degree of viable tumor ($37 \pm 4\%$ vs. $20 \pm 4\%$, $p=0.007$) and lower degree of necrosis ($14 \pm 3\%$ vs. $34 \pm 5\%$, $p=0.007$) than tumors >10cm. No difference in pathologic response was found for tumor type, location, depth, or NCT.

Conclusions: Percent fibrosis showed strong correlation with radiologic response. In contrast, % viable tumor and necrosis showed correlation with inherent aggressiveness of STS. High % fibrosis and low % necrosis were correlated with better OS, and may complement the RECIST criteria in prognosticating patients with STS. We propose incorporating % fibrosis in addition to viable tumor and necrosis as an independent prognostic factor in evaluating post-NRT STS specimens.

27 Loss of Retinoblastoma Protein Expression in Spindle Cell/ Pleomorphic Lipomas and Cytogenetically Related Tumors: An Immunohistochemical Study with Diagnostic Implications

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Background: Spindle cell lipoma is a circumscribed subcutaneous tumor that typically arises on the upper back or neck of older male patients. Spindle cell lipoma is composed of an admixture of adipocytes, short spindle cells, and ropey collagen bundles in variable proportions, sometimes with a myxoid stroma; pleomorphic lipoma is a variant containing floret-like giant cells. Consistent rearrangements of chromosomes 13q and 16q have been identified by cytogenetics. Mammary-type myofibroblastoma and cellular angiofibroma show overlapping histologic features and similar chromosomal losses,

suggesting a possible relationship among these tumor types. The tumor suppressor gene *RB1*, encoding the retinoblastoma (Rb) protein, is located at 13q14, within a minimally deleted region in spindle cell lipoma. The purpose of this study was to examine expression of Rb by immunohistochemistry (IHC) in spindle cell lipoma, pleomorphic lipoma, mammary-type myofibroblastoma, and cellular angiofibroma, as well as histologic mimics, to determine its potential diagnostic utility.

Design: Whole tissue sections of 194 tumors were evaluated: 18 spindle cell lipomas, 20 pleomorphic lipomas, 19 mammary-type myofibroblastomas, 16 cellular angiofibromas, 22 conventional lipomas (8 intramuscular), 18 atypical lipomatous tumors (all positive for MDM2 and CDK4), 19 solitary fibrous tumors, 19 myxoid liposarcomas, 14 hibernomas, 11 deep (aggressive) angiomyxomas, 9 angiomyofibroblastomas, and 9 vulval fibroepithelial stromal polyps. IHC was performed following pressure cooker antigen retrieval using a mouse anti-Rb monoclonal antibody (1:100; G3-245; BD Biosciences). Nuclear staining for Rb was scored as "intact" or "deficient".

Results: Rb expression was deficient in all spindle cell lipomas, pleomorphic lipomas, and cellular angiofibromas and in 17 (89%) mammary-type myofibroblastomas. Rb staining was sometimes difficult to interpret in cellular angiofibromas with reactive stromal changes. Rb was also deficient in 2 (9%) conventional lipomas. Rb expression was intact in all other tumor types evaluated.

Conclusions: Of the soft tissue tumors associated with 13q deletions, all spindle cell lipomas, pleomorphic lipomas, and cellular angiofibromas and most mammary-type myofibroblastomas show loss of Rb expression. Rb expression is intact in histologic mimics. These findings reinforce the pathogenetic relationship among this group of tumors and demonstrate the potential diagnostic utility of IHC for Rb.

28 Complex Interphase Fluorescent In Situ Hybridization Patterns of *EWSR1* Gene in Ewing Sarcoma Using Break Apart Probes

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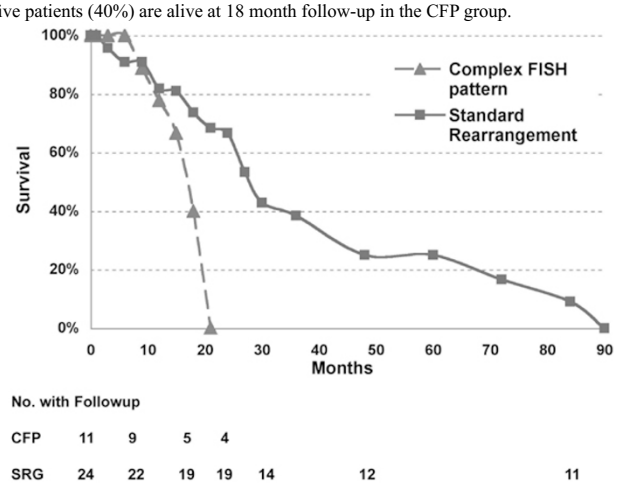
Background: Chromosomal translocations are seen in about one-fourth of all sarcomas. Interphase fluorescent in-situ hybridization (FISH) using break-apart probes has provided the means for widespread use of this technique for sarcoma diagnosis in formalin fixed paraffin embedded tissue. While a typical positive result of a break-apart probe shows one fused signal for normal gene and a split signal indicating the rearranged gene, it is not uncommon to encounter complex FISH patterns (CFP). These include duplications or amplification of native gene and rearranged gene, loss or gain of 5' or 3' sequences and combinations of the above. In this study we retrospectively analyzed the FISH patterns in Ewing Sarcoma.

Design: We collected pathological data and FISH results on Ewing sarcoma cases where FISH was performed for the last 5 years from database of Pathology Department at Memorial Sloan-Kettering Cancer Center. Thirty-six patients were identified. There were 26 males and 10 females. Twenty three cases were primary bone, 10 cases were soft tissue (including 1 kidney, 1 prostate, 1 abdominal cavity) and 3 cases were primary chest wall.

Results: In this group, complex FISH patterns were encountered in eleven out of thirty-six (30%) cases. Majority (8) showed duplication of native *EWSR1* in addition to standard rearrangement (SRG) and/or in association with duplication or loss of 5' or 3' sequences. Of the 11 cases with CFP the male:female ratio was 1.75:1 as compared to 3.2:1 in cases with SRG. The mean age in both groups (CFP and SRG) were 26.4 years and 26.8 years respectively. Metastases at time of presentation was seen in 56% (6/11) in the CFP group and 36% (9/25) in the SRG group. Fourteen out of nineteen patients (74%) were alive at 18 month follow-up in the SRG group whereas two out five patients (40%) are alive at 18 month follow-up in the CFP group.



Conclusions: 1) In addition to standard rearrangement, up to one-third of Ewing Sarcoma patients harbor complex FISH patterns.

2) There is a trend towards high stage presentation in patients with complex FISH patterns.

3) Additional studies with larger number of patients and longer follow-up will aid in understanding the biological significance of these complex FISH patterns.

29 Recurrent t(4;19) Translocation with *CIC-DUX4* Fusion in a Novel Highly Malignant Small Round Cell Soft Tissue Sarcoma

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Background: Translocation-associated sarcomas are defined by distinctive histologic patterns and clinical behavior. Only 8 cases of small round cell soft tissue sarcomas (SRCSTS) harboring t(4;19)(q35;q13.1) have previously been reported, mostly in the pediatric population. In these tumors, *CIC* on chr 19 fuses with *DUX4* on chr 4 resulting in a putative oncogene, *CIC-DUX4*. Herein we describe 4 new cases of SRCSTS harboring this novel recurrent translocation in young adult patients.

Design: We identified 2 initial cases of SRCSTS with complex karyotypes that had t(4;19)(q35;q13.1) translocations. To confirm *CIC-DUX4* fusion and to identify additional cases with t(4;19), we designed RT-PCR primers to detect *CIC-DUX4* fusion and FISH probes to detect *CIC-DUX4* fusion as well as chr 19q13 break-apart. Paraffin blocks from 19 additional cases with similar histologic findings to our initial 2 cases were selected for *CIC-DUX4* testing.

Results: RT-PCR and FISH assays for *CIC-DUX4* fusion and chr 19q13 break-apart confirmed *CIC-DUX4* fusion in our initial 2 cases, which were also negative for Ewings and synovial sarcoma translocations by cytogenetics, RT-PCR and FISH. Of the 19 additional cases, 2 were positive for the *CIC-DUX4* fusion by FISH and negative for *EWS-FLI1* by RT-PCR. The cohort consisted of 2 women (ages 25 & 32) and 2 men (ages 20 & 31). Each presented with a rapidly growing soft tissue mass (mean tumor size 12.8 cm). 2 were located in the thigh, and 1 each in the perineum and calf. All 4 tumors had similar histological features consisting of sheets of primitive small round blue cells with scant cytoplasm, slightly myxoid background, and large zones of necrosis. Only 1 tumor expressed CD99. All 4 patients received neoadjuvant and adjuvant chemotherapy and surgical resection of the primary tumor. 2 had lung metastasis at presentation and a third developed lung and spine metastases 6 months after presentation; all 3 were unresponsive to therapy and died of disease (mean survival 10.6 months). The fourth patient responded to therapy and is alive without evidence of recurrence or metastasis at 27 months.

Conclusions: *CIC-DUX4* primitive SRCSTS represents a novel translocation-associated sarcoma. Our preliminary results suggest aggressive clinical behavior and failure to respond to conventional treatment. Additional FISH and RT-PCR assays are currently being performed for further confirmation and to identify more cases. The results will be reported at the meeting.

30 Advantages of a National Pathological Network for a Systematic Second Review in Sarcomas: Experience of a One-Year Activity in France

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Background: Previous systematic histologic reviews have shown high rate of discordances in sarcomas. Therefore, in 2009 the French Institut National du Cancer (INCa) accredited a network of pathologists to organize a systematic second review of sarcomas.

Design: The network comprises 3 coordinating centers (CC) and 19 referent centers (RC). Every new sarcoma, GIST or desmoid tumor should be referred to one of these centers for a second review. A molecular test should be used for every suspicion of translocation, *MDM2* amplification or specific mutation. This second review is sufficient for GIST with KIT and DOG1 positivity, Kaposi sarcoma with HHV8 positivity, sarcomas with a proved specific translocation, atypical lipomatous tumor-well differentiated liposarcoma (ALT-WDLPS) or dedifferentiated LPS (DDLPS) with a *MDM2* amplification and desmoid tumors with a *CTNNB1* mutation. Other cases even reviewed by a RC are referred to a CC for a third review. The discordant cases are collegially reviewed every month around a multi-head microscope. A set of 30 parameters is collected for every case and registered in a shared database (www.rreps.org).

Results: In 2010, 3653 tumors have been registered with 2357 cases referred from pathologists outside of the network (65%): 1500 cases were spontaneously sent for a second opinion (SO) and 857 were sent for a systematic review according to the recommendations of INCa (SR). The most frequent histotypes were LPS (593 with 226 ALT-WDLPS and 219 DDLPS), undifferentiated pleomorphic sarcomas (532), GIST (508), leiomyosarcomas (368), dermatofibrosarcoma protuberans (184) and desmoid tumors (179). A major discordance has been observed in 25.3% of SO and in 8.1% of SR cases with 220 benign lesions mistaken for a sarcoma, GIST or desmoid tumor (49% of discordances). FISH analysis has been used in 1102 cases with a positive result in 63% and other molecular tests in 961 cases with a positive result in 67%. Frozen tissue has been stored for 885 tumors (24%).

Conclusions: This study confirms the need for a systematic review for sarcomas, GISTs or desmoid tumors. A network organisation improves homogeneity of practice in terms of diagnostic criteria and use of ancillary techniques; knowledge on these rare tumors, and amount and quality of data and material for research purposes.

31 Loss of Heterozygosity, but Not Microsatellite Instability, Is Present in Sporadic Dedifferentiated Liposarcoma: A Study of 46 Genetically Confirmed Cases

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Background: Defective DNA repair mechanisms often lead to complex karyotypes, promote tumorigenesis and are detectible by microsatellite instability (MSI). That such

defects play roles in sarcoma is suggested by the incidence of sarcomas, especially liposarcomas, in patients with germline mutations in mismatch repair genes (Lynch syndrome). Past studies have shown that MSI is rare in sporadic, well-differentiated liposarcomas, but also that the nonlipogenic components of dedifferentiated liposarcomas are genetically more complex than the matched lipogenic components. Thus, DNA repair defects may play a role unique to the dedifferentiated subtype of liposarcoma. This study evaluates the presence of MSI in a large group of well-characterized dedifferentiated liposarcomas.

Design: We studied 46 cases of dedifferentiated liposarcoma, confirmed by histology and 12q13-15 amplification. All cases were sporadic with no documented history of Lynch syndrome. Seven tumors showed low-grade histology. Twenty-five tumors were retroperitoneal with the remainder in the extremities and trunk. The non-lipogenic component and normal tissue from each case were microdissected and the presence of mismatch repair defects was detected using fluorescence based PCR for five mononucleotide microsatellite markers (BAT25, BAT26, NR21, NR24, MONO27) as well as two pentanucleotide repeat markers (PentaC and PentaD). If present, loss of heterozygosity (LoH) at the same markers was also recorded.

Results: Forty-five of 46 (98%) cases of dedifferentiated liposarcoma were microsatellite stable. One case (2%) of high grade dedifferentiated liposarcoma primary to the trunk, showed MSI at a single locus (NR24), and also had LoH at PentaD. Overall, LoH was observed in 10/49 (20%) cases. LoH was only identified at either PentaC (3/49, 6%), PentaD (4/49, 8%) or both (3/49, 6%). The dedifferentiated liposarcomas with LoH showed predominantly (9/10, 90%) high grade pleomorphic histology but the presence of LoH did not correlate statistically with either grade or tumor location (p=0.8 and p=0.4, respectively).

Conclusions: Given the absence of MSI, mismatch repair defects likely do not contribute to sporadic dedifferentiated liposarcoma tumorigenesis. The previously observed association between Lynch syndrome and liposarcoma may represent unique mechanisms in Lynch patients. However, the results do support that LoH at multiple loci are present in a subset of dedifferentiated liposarcomas and may contribute to tumorigenesis or progression.

32 Interobserver Reliability in the Histopathological Diagnosis of Peripheral Cartilaginous Tumors in Patients with Multiple Osteochondromas: How Can We Improve Diagnostic Quality?

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Background: The distinction between benign and malignant peripheral cartilaginous tumors is a challenging subject in surgical pathology.

Design: The aim of this study was to investigate interobserver reliability in histological diagnosis of peripheral cartilaginous tumors in the setting of multiple osteochondromas and to evaluate the most common morphological parameters that characterize each tumor type. Interobserver reliability was assessed by 12 specialized bone-tumor pathologists in a set of 38 cases from patients with multiple osteochondromas.

Results: Substantial agreement in the histological diagnosis of peripheral cartilaginous tumors was observed (intraclass correlation coefficient = 0.78). Evaluation of morphological parameters (singly or collectively) among the concordant cases showed that well-known histological features for malignancy, such as nodularity, presence of binucleated cells, irregular calcification, cystic changes, and necrosis could not reliably distinguish osteochondroma from low-grade peripheral chondrosarcoma. The presence of nuclear pleomorphism and mitoses were useful parameters to differentiate low-grade from high-grade peripheral chondrosarcoma. With regard to cartilage cap thickness among the concordant cases, osteochondromas were significantly thinner than low- and high-grade secondary peripheral chondrosarcomas.

Conclusions: Our study shows that morphological parameters usually associated with malignancy cannot reliably predict neoplastic transformation of an osteochondroma in the setting of multiple osteochondromas and emphasize the importance of a multidisciplinary approach to diagnose peripheral cartilaginous tumors.

33 Extraskeletal Myxoid Chondrosarcoma Presenting as a Primary Bone Tumor: Four Cases with Molecular Confirmation

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Background: The existence of primary bone tumors identical to extraskeletal myxoid chondrosarcoma (EMC) of soft tissue has been controversial due to lack of adequate imaging, histological and molecular documentation. We present 4 cases of EMC in bone that prove it may occur as a primary bone tumor.

Design: Our institutional files were searched for cases of EMC with bone involvement. Imaging features were reviewed by an expert bone radiologist. Clinical and histological features were reviewed to establish the diagnosis, and FISH using break-apart probes for *NR4A3* and *EWSR1* was performed to confirm the diagnosis in all 4 cases.

Results: Four of 128 cases of EMC presented in bone (3%) and were confirmed on imaging studies to be primary bone tumors. Three presented as large intraosseous tumors with cortical breakthrough and soft tissue extension and one was primarily intraosseous (Table 1). Two cases were cellular EMC and 2 classical. *NR4A3* gene rearrangement was found in 3 cases, with one being a variant *NR4A3* rearrangement. *EWSR1* rearrangement was found in 3 cases, including the *NR4A3* negative case that could possibly represent an insertion.

All patients underwent wide resection and 2 adjuvant therapy. Two patients had metastases; one of these died of disease and the other underwent metastasectomy of pulmonary nodules and is currently disease free.

Conclusions: EMC of soft tissue may occur as a primary bone tumor. Failure to recognize this may lead to misdiagnosis. Molecular studies for *NR4A3* and *EWSR1* are important to establish the diagnosis. The biologic behavior and optimal treatment of this rare tumor are not clear at this time and require study of additional cases.

Table 1.

Case	Age (years) Gender	Site	Size (cm)	NR4A3 FISH	EWSR1 FISH	Treatment	Outcome
1	38 M	Ilium, sacrum, paraspinal muscles	12	+	+	Pre-op CTRT, internal hemipelvectomy	NED 5 mo
2	44 M	Mediastinum, manubrium	6.7	-	+	Wide resection, post-op RT	Lung mets, 26 mo
3	54 F	Ilium, gluteus muscles	15	+	+	Pre-op RT, internal hemipelvectomy	NED 36 mo
4	74 M	Anterior 3rd rib	2	+	-	Wide resection	Multiple LR, 36 mo. Lung mets & DOD 61 mo

DOD - dead of disease, LR - local recurrences, mets - metastases, NED - no evidence of disease, RT & CT- radiation and chemo-therapy

34 Metastatic Solitary Fibrous Tumor/Hemangiopericytoma Overexpresses Multiple Growth Factors

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Background: Solitary fibrous tumor (SFT)/ Hemangiopericytoma (HPC) is a mesenchymal tumor of uncertain origin with notoriously unpredictable behavior. Little is known about the signaling pathways underlying tumor progression and metastasis. We studied expression of growth hormones and receptors and their potential prognostic significance in a large series of metastatic and primary SFT/HPC. Comparison with meningeal (CNS) tumors, which are reported to behave more aggressively than in other sites, was also made.

Design: Tissue microarray (TMA) was constructed from 114 tumors from 95 patients, and included 88 soft tissue (ST) SFT/HPCs and 26 CNS HPCs. There were 67 primaries, 39 metastases, and 8 recurrent tumors. Clinical follow-up was available for 55 soft tissue primaries. Immunohistochemistry (IHC) included: IGF1R, EGFR PDGFR-α, PDGFR-β, PDGF-α, PDGF-β, VEGF, cerbB2, ER, and PR. IHC was scored by extent and degree and compared using χ^2 or Fisher exact test.

Results: Compared to primary tumors, metastatic ST tumors had higher expression of growth factors (VEGF, 39% vs. 11%, $p<0.01$; PDGF-β 67% vs. 27% $p<0.001$; PDGF-α, 25% vs. 9%, $p=0.08$) and growth factor receptors PDGFR-α and -β (69% vs. 33%, $p=0.002$; 63% vs. 38% $p=0.046$). Metastases from CNS tumors trended toward elevated levels of PDGFR-α and VEGF only (56% vs. 10%, $p=.057$; 67% vs. 20%, $p=0.07$, respectively). ST primaries trended toward lower expression of both PDGF-α and β, compared to CNS primaries (9% vs. 30%, $p=0.098$, and 27% vs.60%, $p=0.065$). Moderate to high PR was seen in 38% of ST primaries and no CNS primaries ($p=0.024$). No significant difference was seen in IGF1R, EGFR or ER between primary and metastatic disease. All tumors were negative for cerbB2. No significant differences in expression were seen between 9 primary ST tumors which metastasized and 45 that did not.

Conclusions: While growth factor expression was not prognostic in primary tumors, increased expression of multiple growth factors and receptors was seen in metastatic HPC/SFT, suggesting a role in tumor progression. Moreover, CNS primary tumors express more PDGF-α and β than ST tumors, and this may play a role in the more aggressive behavior of these tumors. Targeted therapy against growth factor signaling pathways warrants additional consideration in SFT/HPC. Correlation with histological parameters is on-going.

35 Spindle Cell Liposarcoma, a Distinct Entity or Histologic Variant? Histologic and Molecular Analysis of 12 Cases

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Background: Spindle cell (SC) liposarcomas (LPS), an incompletely characterized tumor, has been considered toa variant of myxoid LPS, well differentiated LPS or SC lipoma. Using strict morphologic criteria we analyzed a series of cases of SC-LPS to determine if clinical and molecular characteristics warrant its acceptance as a unique entity.

Design: Cases classified as "spindle cell LPS" or "low-grade LPS with spindle cell features" were identified from the authors' consultation and hospital files and reviewed by at least 3 authors. Diagnosis was based on 1) a low grade, monotonous SC component and 2) a population of typically mono- or binucleated lipoblasts with an "ice cream cone" appearance. CGH analysis and FISH for the DDIT3 fusion and MDM2 amplification were performed. A panel of immunohistochemical stains, including HMGA2, MDM2, CDK4, desmin, SMA, myogenin, MyoD1, S100 protein, CD34, and Ki-67, was evaluated. Clinical follow-up was obtained.

Results: Of 25 initially identified cases, 12 cases clearly met morphologic criteria; 6 cases were reclassified as low grade sarcoma, not further classified. CGH analysis of ambiguous cases excluded 5 cases as low-grade dedifferentiated LPS and 2 cases as SC lipoma. SC-LPS affected the sexes equally (6M:6F). Patients ranged from 15 to 82 years

(mean 47). Tumors arose in the lower extremity (5), groin/vulva/paratesticular tissue (4), inguinal area (1), abdominal wall (1) and shoulder (1). Tumors ranged in size from 2 to 20 cm (mean 7.7 cm). CGH showed no gains or losses. No tumors showed MDM2 amplification or the DDIT3 fusion by FISH. By immunohistochemistry, the SC-LPS expressed CD34 (10/12), S100 protein (7/12) and desmin (3/12); other antigens were negative. Proliferation was low (Ki-67 1-3%). Clinical follow-up was obtained for 10 patients (range, 9-113 mos; mean, 47mos): 1 patient developed bone metastasis at 111 mos but is alive with disease; the remaining patients had no recurrence or metastasis.
Conclusions: Spindle cell liposarcoma is a distinct form of liposarcoma having a characteristic morphologic appearance, a flat CGH profile and indolent behavior. Since late metastases can be seen, long term follow up is needed to assess full metastatic potential. Some features of SC-LPS overlap with low-grade DDL, which is clinically more aggressive, and SC lipoma, which is benign, indicating the need for molecular-genetic studies in histologically ambiguous cases.

36 MUC4 Is a Sensitive and Specific Marker for Sclerosing Epithelioid Fibrosarcoma: Association with *FUS* Gene Rearrangement

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Background: Sclerosing epithelioid fibrosarcoma (SEF) is a rare aggressive fibroblastic neoplasm composed of cords of epithelioid cells embedded in a dense collagenous stroma. The reported immunophenotype of SEF is non-specific. Some SEF cases show morphologic and molecular overlap with low-grade fibromyxoid sarcoma (LGFMS), suggesting a relationship between these tumor types. MUC4 has recently been identified as a sensitive and specific marker for LGFMS; MUC4 expression was also observed in 2 tumors with hybrid features of SEF and LGFMS. We investigated MUC4 expression in SEF and other epithelioid soft tissue tumors to determine (1) the potential diagnostic utility of MUC4 for SEF and (2) the association between MUC4 expression and *FUS* rearrangement in SEF.
Design: Whole sections of 146 tumors were evaluated: 34 SEF cases (8 hybrid LGFMS/SEF), 20 epithelioid sarcoma, 11 clear cell sarcoma, 11 metastatic melanoma, 10 PEComa, 10 alveolar soft part sarcoma, 10 epithelioid angiosarcoma, 10 epithelioid hemangioendothelioma, 10 epithelioid gastrointestinal stromal tumor (GIST), 10 myoepithelial carcinoma (MEC), and 10 biphasic synovial sarcoma (B-SYS). Immunohistochemistry was performed following antigen retrieval using a mouse anti-MUC4 monoclonal antibody (1:500; 8G7; Santa Cruz). FISH was performed on 33 SEF cases using *FUS* break-apart probes (Abbott).
Results: Strong diffuse cytoplasmic staining for MUC4 was observed in 25 of 34 (74%) SEF cases, including all 8 hybrid tumors. *FUS* rearrangement was found in 7 of 20 (35%) MUC4-positive SEF cases with successful FISH. The prevalence of *FUS* rearrangement was similar in hybrid LGFMS/SEF and SEF cases without an LGFMS component. *FUS* rearrangement was not detected in any MUC4-negative SEF cases. MUC4 expression was also seen in 9 of 10 (90%) B-SYS cases, predominantly in the glandular component. All other tumor types were negative for MUC4, apart from focal reactivity in 2 epithelioid GISTs and 1 MEC.
Conclusions: MUC4 is a sensitive and relatively specific marker for SEF. MUC4 expression correlates with glandular epithelial differentiation in B-SYS and is very limited in other epithelioid soft tissue tumors. These findings suggest that SEF are biologically heterogeneous. MUC4-positive SEF with *FUS* rearrangement are likely closely related to LGFMS. MUC4-positive SEF that lack *FUS* rearrangement may be related to LGFMS but could have alternate gene fusions. SEF without MUC4 expression probably represent a distinct group of tumors.

37 Histidine Decarboxylase Expression by Immature Myeloid Cells May Affect Peak Bone Density in Mice

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Background: Our group has identified a major gene on chromosome 2 in mice that is associated with variation in peak bone mineral density and femoral bone strength. Variation in this gene may explain why C57BL/6 mice have significantly less bone density than DBA/2 mice. Taking advantage of the synteny between human genome sequence and the murine introgressed region on chromosome 2, we identified histidine decarboxylase (HDC), the rate-limiting enzyme in the biosynthesis of histamine, as a positional candidate gene for the observed variation in bone density. Notably, others have reported HDC knockout mice have increased bone formation and conversely increased HDC expression has been reported in circulating monocytes from patients with osteoporosis. We hypothesized that variance in HDC expression between C57BL/6 and DBA/2 mice may account for observed differences in bone density.
Design: Fresh femoral bone tissue samples were prepared from at least six adult C57BL/6 and six adult DBA/2 mice of each gender. Portions of each sample were prepared for nucleic acid and protein extraction, tissue embedding for immunohistochemistry, and subsequent cell sorting by flow cytometry (FACS). HDC expression was analyzed by real-time RT-PCR and Western blot analysis. Expression was localized by immunostaining for HDC (Progen) and mast cell contamination was excluded using CD117.
Results: C57BL/6 mice showed significantly increased marrow HDC expression compared with DBA/2 mice. We did not observe significant gender differences. Immunohistochemical staining suggested the source of HDC may be immature myeloid cells (IMCs). In turn, we have purified CD11b+Ly6G+ IMCs with low side scatter using FACS for future HDC expression and promoter methylation analysis.
Conclusions: Our data suggest histamine physiology in the bone marrow could be an important determinant of bone density. Moreover, we suspect that inherited variation in the HDC enzyme's promoter activity may explain some of the natural variation in peak bone density.

38 CD47 Is a Therapeutic Antibody Target in Leiomyosarcoma

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Background: Leiomyosarcoma (LMS) is a neoplasm of smooth muscle for which limited therapeutic options exist. Tumor-associated macrophages (TAMs) can act as promoters of cancer progression; we have shown that in LMS a high density of TAMs is associated with poor patient outcome. CD47 is a transmembrane protein that is highly expressed on tumor cells and that binds to SIRPa on macrophages. The interaction between CD47 and SIRPa prevents macrophages from phagocytosing tumor cells. Inhibition of this interaction with CD47-specific monoclonal antibodies (mcAbs) allows phagocytosis to occur and decreases tumor burden in experimental models of leukemia and lymphoma.
Design: mRNA profiling and immunofluorescence staining for CD47 was performed on normal uterus, leiomyomas, and LMS samples. In vitro phagocytosis assays using 2 human LMS cell lines and human or mouse macrophages tested the ability of anti-CD47 mcAbs to enable phagocytosis of LMS cells. Xenotransplants of 2 human LMS cell lines in mice assessed the effect of anti-CD47 mcAbs on primary tumor growth. A model for neoadjuvant treatment was developed by starting anti-CD47 treatment one week prior to resection of primary subcutaneous LMS tumors and allowing mice to develop lymph node and lung metastases; the efficacy of anti-CD47 therapy was evaluated in this model.
Results: We show that CD47 is present on LMS tumor cells at a higher level than on benign leiomyomas and normal muscle, and that anti-CD47 mcAbs enable phagocytosis of 2 human LMS cell lines by human and mouse macrophages in vitro. Treatment with anti-CD47 mcAbs significantly inhibits primary tumor growth of 2 human LMS cell lines transplanted in mice. We developed a model for neoadjuvant therapy of LMS in which mice develop lymph node and lung metastases after resection of their primary tumors. In this model, anti-CD47 treatment increases recurrence-free survival and significantly diminishes the size and incidence of lymph node and lung metastases.
Conclusions: These results demonstrate that anti-CD47 mcAb therapy may be a promising treatment for LMS and that targeting CD47 on LMS cells has the potential to change the behavior of resident TAMs to inhibit, rather than promote, tumor growth.

39 Clinicopathological and Prognostic Significance of Akt-mTOR and MAPK Pathways and Antitumor Effect of mTOR Inhibitor in Malignant Peripheral Nerve Sheath Tumor

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Background: Malignant peripheral nerve sheath tumor (MPNST) is a chemotherapy-resistant sarcoma showing a poor prognosis. A novel effective antitumor drug for MPNST is desired, but it has not been found yet. Akt-mTOR and MAPK signaling pathways are known to be activated in various types of cancer, and some kinds of mTOR inhibitors are available in clinical practice. However, the clinicopathological and prognostic significance of activation of Akt-mTOR and MAPK pathways in MPNSTs have yet not been revealed. Additionally, antitumor efficacy of mTOR inhibitor in MPNST has not been investigated well.
Design: We investigated the activation status of Akt-mTOR (Akt, mTOR, p70S6K, S6RP, 4E-BP1, HIF-1α) and MAPK (Erk1/2) pathways in 129 MPNST formalin-fixed paraffin-embedded (FFPE) samples from 99 patients by immunohistochemistry (IHC). Five samples, for which frozen material was available, were also investigated by Western blotting (WB). The antitumor effect of mTOR inhibitor (Everolimus) was examined using 6 MPNST cell lines (FU-SFT8611, FU-SFT9817, HS-sch-2, HS-PSS, YST-1, FMS-1) by CCK-8 cell viability assay, Matrigel invasion assay and wound healing assay.
Results: Immunohistochemically positive expressions of phosphorylated-Akt (p-Akt), p-mTOR, p-p70S6K, p-S6RP, p-4E-BP1, HIF-1α and p-Erk1/2 were observed in 59.7%, 48.8%, 62.8%, 54.3%, 62.8%, 74.4% and 72.9% of MPNST samples, respectively. The expression levels of p-Akt and p-mTOR by WB corresponded closely to the levels observed by IHC. Clinicopathological examination showed that activation of Akt-mTOR and MAPK pathways was frequently observed in the subgroups of deep location, frequent mitoses, high MIB-1 labeling index and high histological grade. Prognostic analysis revealed that activation of Akt-mTOR pathway was significantly associated with poor prognosis, but activation of MAPK pathway did not influence the prognosis. Experiments with MPNST cell lines showed that Everolimus caused concentration-dependent inhibition of MPNST cell proliferation. Everolimus also inhibited cell invasion and cell migration at a lower drug concentration achieved clinically.
Conclusions: Activation of Akt-mTOR pathway is associated with malignant progression and poor prognosis in MPNST. mTOR inhibition by Everolimus shows antitumor effect in MPNST cell lines and can be a candidate therapeutic target in MPNST.

40 Karyotyping Myofibroblastic/Fibroblastic Tumors: Continuing Usefulness of Standard Cytogenetic Methods in Detecting Novel Genetic Findings and Confirming the Histologic Diagnosis

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Background: Myofibroblastic/Fibroblastic tumors encompass a broad variety of benign to borderline to malignant tumors. The category continues to undergo change as tumors are more accurately classified. Because this is a large, diverse category, karyotypic

information is limited. Classic cytogenetics is still helpful in providing first leads to uncover molecular level abnormalities. However, artifacts of long term in-situ culture must be recognized and threshed from the harvest of findings.

Design: Karyotypes of myofibroblastic/fibroblastic tumors (M-FT) according to W.H.O. 2002 classification and recently proposed M-FT were searched for in the cytogenetics database at our institution for 2001-2011 (to October 1). Twelve categories of tumors were identified. Tumors were stratified according to: 1) successful culture, 2) normal karyotype (possible fibroblast overgrowth), 3) abnormal clonal karyotype expected for the histologic diagnosis, and 4) novel abnormal karyotype. Percent successful karyotype per tumor type and percent non-clonal cell lines were calculated. Length in time in culture and type of specimen (biopsy, resection, re-exision) were noted in conjunction with tumor diagnosis, location, size, grade, and stage.

Results: The results are provided in table form..

MYOFIBRO-FIBROBLASTIC TUMOR KARYOTYPES

TUMOR	NUM-BER	NUMBER SUCCESSFULLY KARYOTYPED	NUMBER WITH NORMAL KARYOTYPE	NUMBER WITH EXPECTED KARYOTYPE	NUMBER WITH NOVEL ABNORMAL KARYOTYPE	PERCENT WITH ABNORMAL KARYOTYPE
NODULAR FASCIITIS	8	6	4	0	2	33%
DESMOID TUMOR	23	19	16	3	0	16%
CALCIFYING APONEUROTIC FIBROMA	2	1	0	0	1	100%
DFSP	5	5	3	0	2	60%
FIBROSARCOMA ARISING IN DFSP	2	2	0	0	2	100%
INFLAMMATORY MYOFIBROBLASTIC TUMOR	2	2	1	1	0	50%
LOW GRADE FIBROMYXOID SARCOMA	9	7	2	2	3	71%
SCLEROSING EPITHELIOID FIBROSARCOMA	1	1	0	1	0	100%
MYOFIBROSARCOMA	3	2	0	0	2	100%
MYXOFIBROSARCOMA	23	21	8	13	0	62%
DESMOPLASTIC FIBROBLASTOMA	1	1	0	0	1	100%
MYOFIBROBLASTIC/ FIBROBLASTIC TUMOR NOS	9	6	3	0	3	50%

Fifty percent of cases exhibited secondary non-clonal abnormalities; 28% of these "non-clonal" changes were closely related to the clonal change, reproduced in subsequent specimens as "non-clonal" or showed abnormalities consistent with the clinical history and morphology.

Conclusions: While expensive and labor intensive, in selected circumstances, judiciously examined, traditional karyotypes can provide new information helpful in evaluating the pathogenesis of and relationships among myofibroblasti/fibroblastic tumors.

41 Solitary Fibrous Tumor: Is There a Molecular Relationship with Cellular Angiofibroma, Spindle Cell Lipoma and Mammary-Type Myofibroblastoma?

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Background: Solitary fibrous tumor (SFT) is a distinct mesenchymal tumor characterized by ovoid to spindled cells, characteristic thick-walled branching ("staghorn") blood vessels, stromal hyalinization, variable amounts of lipomatous differentiation and immunoreactivity for CD34. Recent studies have shown loss of 13q in a group of morphologically similar entities including cellular angiofibroma, mammary-type myofibroblastoma and spindle cell lipoma. The histologic and immunophenotypic overlap between solitary fibrous tumor and the latter group of tumors has been recognized, raising the possibility that all four of these tumors may be genetically linked.

Design: We tested a group of 40 SFTs including some malignant SFTs to assess for loss of *RB1* (13q14) by fluorescence in situ hybridization (FISH). Additionally, a group of cellular angiofibromas (1 case), spindle cell lipomas (6 cases) and mammary-type myofibroblastomas (4 cases) were analyzed as a control group.

Results: All cases (38/38) of solitary fibrous tumor with evaluable signals failed to show loss of *RB1* (13q14) by FISH while all cases of cellular angiofibroma (1/1), spindle cell lipoma (6/6) and mammary-type myofibroblastoma (4/4) showed either monoallelic or biallelic loss of *RB1*.

Conclusions: Although solitary fibrous tumor may share overlapping morphologic and immunophenotypic features with cellular angiofibroma, mammary-type myofibroblastoma and spindle cell lipoma, the absence of *RB1* loss suggests that they are not related genetically.

42 Bone and Soft Tissue Pathology Discovered in Bone Bank Donors: An Analysis of 109 Lesions

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Background: Bone is the second most frequently transplanted human tissue with bone allografts being used in orthopaedics. Bone banking generally focuses on eliminating risk of infection and selecting bone with structural integrity. Screening procedures aim to exclude donors at risk of transmitting a malignant neoplasm. However, bone and soft tissue lesions are still identified during the procurement process. The incidence and significance of such lesions is uncertain. We analyze a large series of bone and soft tissue lesions sent for pathologic examination by a large regional tissue bank.

Design: We examined bone and soft tissue cases from anonymous post mortem donors submitted for review by a regional tissue bank from 2002-2011. The selected tissue was deemed abnormal by the tissue bank based on visual inspection or roentgenographic examination. Gross and microscopic examination was performed by an experience bone and soft tissue pathologist to ascertain the viability and safety of the donor's unaffected skeletal tissues. All H&E slides were re-reviewed for this study.

Results: 109 lesions were identified in 89 donor patients. 71% of donors were male and 29 female. Most commonly affected bones in descending order included femur, tibia, patella, humerus, fibula, costal cartilage, and ilium. Bone lesions included bone infarct (16), osteopenia (12), traction exostosis (8), bone island (5), enchondroma (5), intraosseous ganglion cyst (1), osteochondroma (3), non-ossifying fibroma (2), hemangioma (1), solid ABC (2), subchondral cyst (1), subchondral fracture (1), and osteocartilaginous loose body (1), non-specific remodeling (4), reactive/degenerative changes of bone and cartilage (3). The most common soft tissue sites included Achilles tendon, patellar tendon and soft tissue around the knee. Soft tissue lesions included tendon with degenerative changes (12), synovial cyst (4), ganglion cyst (4), lipoma (2), metaplastic bone (2), tenosynovial giant cell tumor (1), gout (1) and calcific tendinosis (1). 16 lesions identified by the bone bank had no pathological abnormality identified.

Conclusions: Bone and soft tissue lesions discovered during bone banking procurement procedures are uncommon and invariably benign. Bone infarct and osteopenia were the most common bone lesions discovered. The most common soft tissue lesion was degenerative changes of tendons and ganglion and synovial cysts. The most common bone tumors were enchondroma and osteochondroma. Unaffected donor bone can be utilized in the vast majority of cases. However, cases of osteopenia raise concern for the quality of bone procured.

43 NY-ESO-1, a Cancer/Testis Antigen, Is Differentially Expressed in Myxoid/Round Cell Liposarcomas Compared to Other Liposarcoma Subtypes and Myxomatous Neoplasms

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Background: NY-ESO-1 is a highly immunogenic cancer/testis antigen (CTA) expressed in testicular germ cells and in various cancers where it can induce cellular and humoral immunity. Immunotherapy targeting this antigen has shown promise in clinical trials involving NY-ESO-1 expressing tumors, including synovial sarcomas, melanomas and solid organ tumors. Liposarcomas (LPS) are common soft tissue sarcomas, and surgery with or without chemoradiation therapy remains the main stay of treatment. Gene expression studies have shown upregulation of CTAs in LPS, particularly the myxoid/round cell (MRCL) subtype. Herein, we evaluated the expression of NY-ESO-1 antigen among a variety of lipomatous and related myxomatous neoplasms by immunohistochemistry (IHC) using a monoclonal antibody to NY-ESO-1.

Design: Formalin-fixed paraffin-embedded (FFPE) blocks were obtained for lipomas (n=19; 3 with myxoid change) and the following LPS subtypes (n=44): MRCL (n=18); well-differentiated (n=10; 2 with myxoid change); dedifferentiated (n=10); and pleomorphic (n=6). FFPE blocks were also retrieved for the following myxomatous neoplasms (n=15): low grade fibromyxoid sarcoma (LGFS, n=5); myxofibrosarcoma (MFS, n=5); and myxoma (n=5). Utilizing standard IHC staining protocols, full sections were stained with NY-ESO-1 (clone E978; Santa Cruz Biotechnology). Staining was assessed for intensity (1-3+), percentage of tumor positivity and location.

Results: 16/18 (89%) MRCL expressed NY-ESO-1. The majority demonstrated uniform staining (>75% positive cells) of moderate to strong intensity (2-3+), while two cases showed a more heterogenous pattern: 3+ intensity in 60% of cells and 1+ intensity in 20% of cells. 3/6 (50%) pleomorphic LPS demonstrated a range of staining: 1+ intensity in 50% of cells; 2+ intensity in 5% of cells; and 3+ intensity in 90% of cells. One (10%) case of dedifferentiated LPS showed strong, diffuse staining (3+ intensity in 75% of cells). In all positive instances, both nuclear and cytoplasmic staining was present. There was no immunoreactivity seen in lipomas, well-differentiated LPS, LGFS, MFS or myxomas.

Conclusions: Our data indicate that NY-ESO-1 is expressed with high frequency in MRCL and can be of diagnostic utility within the differential diagnosis, which includes other myxoid neoplasms, such as well-differentiated LPS with myxoid change, soft tissue myxomas and MFS. Furthermore, the high level of NY-ESO-1 expression in MRCL enables the potential use of targeted immunotherapy in the treatment of this malignancy.

44 Aberrant Calreticulin Expression Is Involved in the Dedifferentiation of Dedifferentiated Liposarcoma

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Background: Liposarcomas are a representative group of soft tissue sarcomas with variably hampered adipogenesis, which is most exemplified by its dedifferentiated subtype. Despite some characteristic genetic alterations harbored in the subtype, the factors responsible for inhibiting the adipocytic differentiation remains unknown. A recent gene expression profiling study has identified several unique genes that are highly expressed in dedifferentiated liposarcoma, and the gene encoding calreticulin, a major Ca^{2+}-buffering protein that can inhibit adipocytic differentiation, was found to be overexpressed.

Design: We investigated the expression of calreticulin in 45 liposarcomas including 15 dedifferentiated tumors at both the protein and mRNA levels, and compared the levels to those in lipomas and normal fat. The anti-adipogenic function of calreticulin was assessed using a dedifferentiated liposarcoma cell line, FU-DDLS-1, by transfection of siRNA. Fluorescence in situ hybridization (FISH) and quantitative reversed transcription-polymerase chain reaction (RT-PCR) for putative microRNAs (miRNAs) targeting calreticulin were performed to address potential genetic and epigenetic alterations in the calreticulin gene (CALR).

Results: Immunohistochemically, calreticulin was consistently expressed in dedifferentiated areas of dedifferentiated liposarcomas and commonly in atypical stromal cells and/or lipoblasts in their well differentiated areas (87%), whereas large

vacuolated adipocytic cells in the tumors or normal fat were essentially negative. The results were further supported by the findings of Western blotting and quantitative RT-PCR analyses. Although abnormalities in 19p13.1-13.2, where CALR is localized, were uncommon in the dedifferentiated liposarcomas examined by FISH, the expression of miR-1257, which was extracted by a computational prediction software program, was significantly suppressed in the dedifferentiated subtype. The downregulation of calreticulin by siRNA could induce adipogenesis in dedifferentiated liposarcoma cells and reduce the cell proliferation.

Conclusions: Our results suggest that calreticulin is aberrantly expressed in dedifferentiated liposarcoma and is involved in its dedifferenitation and/or tumor progression.

45 Network of Thick Fibrils in Normal Fetal and Chondrodysplastic Articular Cartilage

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Background: Articular cartilage is a highly specialized connective tissue consisting of sparse chondrocytes widely dispersed in an abundant hyaline matrix. How this complex structure is being formed and how the discrete chondrocytes communicate with each other are not well understood. An interlacunar network and thick fibrils has been described in developing hyaline cartilage, the nature of which is not well characterised. The functions of the network and fibrils remain speculative. This study attempts to further characterise the thick fibrils in fetal articular cartilage and to describe their changes in cases of type II achondrogenesis and chondrodysplasia with giant chondrocytes.

Design: Articular cartilage from normal and chondrodysplastic fetuses were obtained at autopsies and processed for light and electron microscopy. Light microscopic sections by H&E, and Victoria blue with/without hyaluronidase pre-digestion. For electron microscopy, specimens were fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer at pH 7.4 with/without addition of 0.5% tannic acid, postfixed in 1% osmium tetroxide in similar buffer with/without !.5% potassium ferrocyanide.

Results: Numerous straight or slightly curved blue fibrils were seen traversing the matrix. Some fibrils were seen attached to chondrocytes and occasionally formed connections between two cells. In Type II achondrogenesis, the thick fibrils were less distinct being blurred in a smudged background. Some fibrils appeared twisted and had a spiral appearance. A network of thick fibrils were often seen surrrounding the giant chondrocytes.

In tannic acid-glutaraldehyde fixed cartliage, the thick fibrils appeared as bundles of 10-12 nm diameter microfibrils. The microfibrils bundles were coated by a variale amount of amorphous materials. These bundles were often seen oriented in the same direction as the surrounding colllagen fibril. Some thick fibrils were seen attached to chondrocytes in apparent continuity with intracytoplasmic filaments. The cellular attachments of thick fibrils were more frequenly encountered in giant cell chondrodysplasia.

Conclusions: The thick fibrils in articular cartilage are primarily bundles of 10 nm microfibrils microfibrils coated by matrix proteins. They are directly attached to chondrocytes resulting in an interconnecting network which may play an organisational role in the formation of the articular cartilage.

46 Chordoma Arising in Benign Notochordal Cell Tumor: A Detailed Radiological, Gross and Microscopic Description of Three Cases Involving the Lumbar Spine

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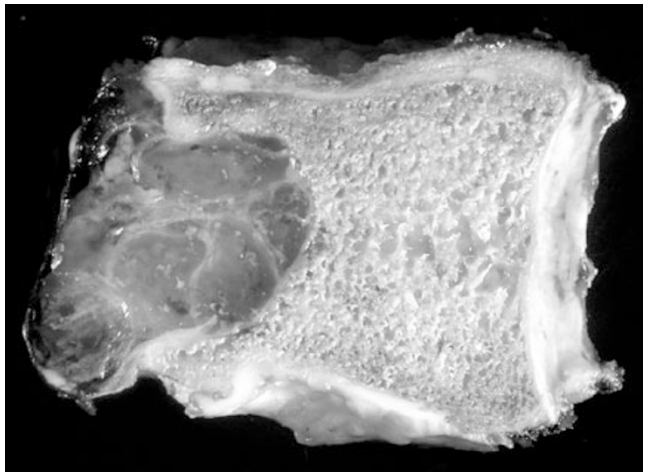
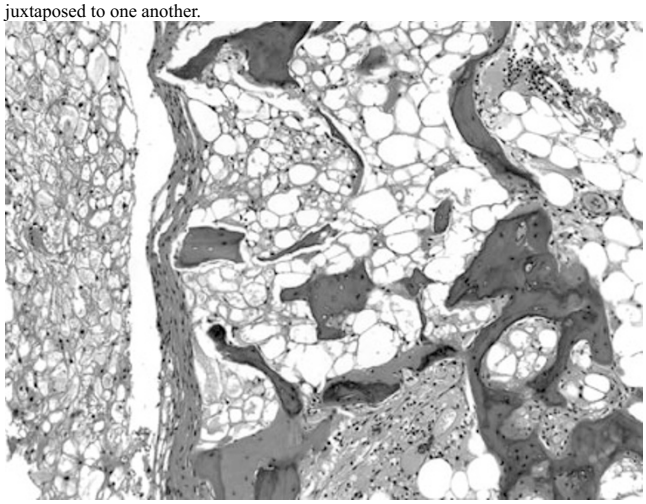
Background: Chordoma is a rare primary malignant bone neoplasm that recapitulates the emberyonic notochord. It arises almost exclusively in the axial skeleton with predilection for its proximal and distal segments. The pathogenesis of chordoma is still unknown, however, some investigators have suggested that it develops from benign notochordal cell tumor (BNCT) based on demonstrating both lesions in the same patient or by illustrating that they have the same anatomical distribution.

Design: We identified from the surgical pathology files of the department of pathology of Massachussettss General Hospital and the consultation files of one of the authors three cases of chordoma arising directly adjacent to BNCT. The radiological, gross and microscopic features were carefully analyzed.

Results: The three tumors arose in two women and one man and their ages were 42,17 and 31 respectively. On imaging studies the BNCT manifested as an irregular region of sclerosis located within the center of the vertebral body and the chordoma appeared as an oval area of lytic destruction that was centered posterior to the BNCT andtransgressed the cortex and extended into the spinal canal.



Microscopically, both lesions demonstrated characteristic histologic features and were juxtaposed to one another.



Conclusions: Hypotheses regarding the etiology of chordoma have largely been speculative with very few published examples of both lesions intimately related to one another. Our study clearly demonstrates the anatomic proximity of the two neoplasms provides further evidence that BNCT is the precursor lesion for chordoma.

47 FBXW7 Mutations in a Subset of Embryonal Rhabdomyosarcoma

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Background: Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in children under 15 years of age. Histologic subtypes correlate not only with prognosis, but also with well-described cytogenetic abnormalities. Little is known, however, concerning the frequency of mutations in RMS of cancer genes known to play a significant role in other malignancies.

Design: Thirty archival rhabdomyosarcomas were reviewed and histologically sub-classified. There were 13 embryonal RMS, 8 alveolar RMS and 9 unclassified. Cytogenetic information available from the time of diagnosis was also compiled. DNA was prepared from formalin fixed, paraffin-embedded tumor tissue (>90% tumor), and screened for mutations using a multiplexed assay panel with a mass spectrometry-based readout that covers 390 mutations across 30 cancer genes.

Results: Mutations in one or multiple cancer genes were observed in 5 of the 30 cases (17%) and were seen only in the embryonal subtype (5/12, 41.7%). Of note, a FBXW7 R465C and a FBXW7 R505C mutation, not previously described in rhabdomyosarcomas, was present in two of the embryonal cases. Additional mutations included NRAS Q61H and Q61P (2/30, 6.7% of all cases; 2/12, 16.7% of embryonal RMS), KRAS G12C (1/30, 3.3%; 1/12, 8.3%) and FGFR4 V550M (1/30, 3.3%; 1/12, 8.3%), all of which have been previously described in RMS. No mutations were observed in any of the alveolar RMS (0/8), irrespective of cytogenetic abnormalities.

Conclusions: FGFR4 is a potential treatment target in embryonal RMS, and routine genotyping of this subset of tumors could help identify patients for clinical trials. FBXW7 is a member of the F-box family and serves as part of the ubiquitin protein ligase complex that regulates degradation of MYC, cyclin E and MCL1. The finding of recurrent mutations in this gene suggests a significant role in deregulation of gene transcription, apoptosis, and the cell cycle in embryonal RMS.

48 SOX11 Is a Marker for Myxoid/Round Cell Liposarcoma

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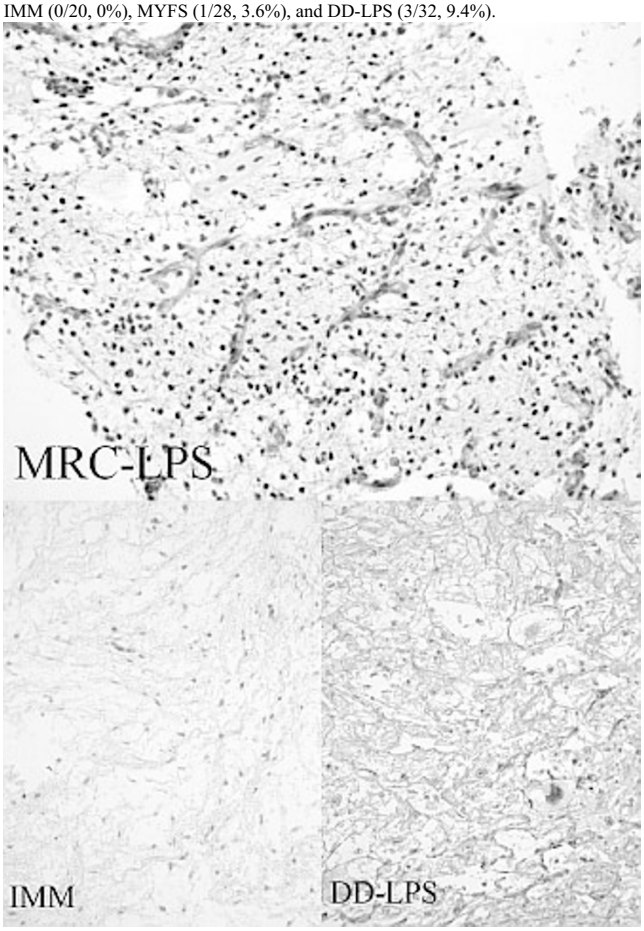
Background: Histologic classification of myxoid lesions by histology alone can pose a diagnostic challenge, especially with limited biopsy material. We hypothesized that publicly available gene expression microarray databases may yield candidate genes of potential value in discriminating between myxoid neoplasms. This bioinformatics-based approach indeed suggested that the transcription factor SOX11 (Sry-related HMG-box-11) is highly expressed by myxoid liposarcomas. With the goal of more accurate diagnosis of these lesions, we therefore evaluated the reactivity of SOX11 in a range of myxoid neoplasms: myxoid/round cell liposarcoma (MRC-LPS), intramuscular myxoma (IMM), myxofibrosarcoma (MYFS), and dedifferentiated liposarcoma (DD-LPS).

Design: A total of 18 MRC-LPS, 20 IMM, 28 MYFS, and 32 DD-LPS were retrieved and reviewed. Tissue microarrays (TMAs) were constructed in duplicate. TMAs, as well as whole sections, were evaluated for immunoreactivity to SOX11 (Sigma, rabbit, HPA000536, 1:100). Independent scoring by two of the investigators (KJJ and JHH) for the percentage of tumor cells demonstrating nuclear reactivity and intensity of expression (score 0, 1, 2, 3) yielded an immunohistochemistry score (0-300). A threshold for positive reactivity was set at an IHC score of 100.

Results: Nearly all MRC-LPS cases (16/18, 89%) demonstrated positive immunoreactivity for SOX11. SOX11 immunoreactivity was absent to rare in cases of IMM (0/20, 0%), MYFS (1/28, 3.6%), and DD-LPS (3/32, 9.4%).



The positive SOX11 marked the monster cells in one MYFS case and the areas of high grade DD-LPS cases. SOX11 immunoreactivity was negative or decreased within the maturing adipocytic component of MRC-LPS tumors. Normal fat was negative.

Conclusions: 1) Publicly available gene expression microarray databases may be a source for markers of potential diagnostic use; 2) SOX11 transcription factor is frequently expressed in myxoid liposarcomas; 3) SOX11 is absent to rare in myxomas, myxofibrosarcomas, and dedifferentiated liposarcomas; 4) Thus, this differential immunoreactivity strongly suggests SOX11 immunohistochemistry has potential diagnostic utility for the diagnostic evaluation of myxoid lesions, including small needle biopsy material.

49 Diagnostic Value of aP2/FABP4 Expression in Soft Tissue Tumours

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Background: FABP4 (fatty acid-binding protein-4), also known as aP2 (adipocyte P2), is a fatty acid binding protein found in the cytoplasm of adipocytes. We have previously shown that aP2/FABP4 is useful in detecting lipoblasts using monoclonal and polyclonal antibodies. In this study, using a commercially available polyclonal antibody, we examined the diagnostic utility of aP2/FABP4 expression in a wide range of soft tissue tumours.

Design: Expression of aP2/FABP4 was determined by indirect immunoperoxidase immunohistochemical staining with the polyclonal antibody (Sigma, HPA002188), in paraffin sections of (formalin-fixed) normal adipose tissue and a wide range of soft tissue tumours.

Results: Fat cells in normal adipose tissue and lipomas (n=21) showed aP2/FABP4 expression around cytoplasmic fat. Some capillary endothelial cells also expressed aP2/FABP4, but fibroblasts, muscle and other cell types were negative. Other benign adipocytic tumours, hibernomas (n=3) and lipoblastomas (n=5) showed strong aP2/FABP4 staining of brown fat cells and lipoblasts respectively. Spindle cell lipomas (n=5) showed positive staining of fat cells but not spindle cells. In intermediate and malignant adipocytic tumours, the antibody labelled lipoblasts in atypical lipomatous tumour/well differentiated liposarcoma (n=26), myxoid/round cell liposarcoma (n=8) and pleomorphic liposarcoma (n=3). aP2/FABP4 expression was not seen in myxoid and other soft tissue tumours showing myxoid change containing (lipoblast-like) vacuolated cells including myxoma (n=10), low grade fibromyxoid sarcoma (n=2), myxofibrosarcoma (n=10), ossifying fibromyxoid tumour (n=2), leiomyosarcoma (n=2), rhabdomyosarcoma (n=3), solitary fibrous tumour (n=4) and chordoma (n=2).

Conclusions: aP2/FABP4 expression is seen in mature and immature fat cells. Expression of aP2/FABP4 aids in the identification of fat cells and lipoblasts in adipocytic tumours and is particularly useful in distinguishing myxoid/round cell and pleomorphic liposarcomas from myxoid (eg myxofibrosarcoma), spindle cell (eg leiomyosarcoma) and pleomorphic (eg rhabdomyosarcoma) sarcomas that contain vacuolated tumour cells which morphologically resemble lipoblasts.

50 Molecular Distinction of Chondrosarcoma from Chondroblastic Osteosarcoma through *IDH12* Mutations

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Background: Distinguishing chondrosarcoma from chondroblastic osteosarcoma particularly on a biopsy specimen can be difficult and highly subjective. This distinction is critical in determining the most appropriate treatment modality, since adjuvant chemotherapy is standard treatment for osteosarcoma while chondrosarcoma is generally treated by surgical excision alone. Cartilaginous neoplasms have recently been shown to frequently (56%) harbor mutations in the metabolic enzymes IDH1 and IDH2 (*IDH1* > *IDH2*), whereas a wide range of other mesenchymal tumors have not. The purpose of this study was to analyze if the presence of *IDH1/2* mutations can be used to distinguish between chondrosarcoma and chondroblastic osteosarcoma.

Design: 7 predominantly high grade chondrosarcoma tumors and 19 osteosarcoma tumors (12 purely chondroblastic, 7 mixed chondroblastic) were reviewed and DNA was extracted from formalin-fixed, paraffin-embedded tissue. Mutational analysis using a multiplexed PCR genotyping platform was used to query for hotspot mutations in the genes *IDH1* at codon R132 and *IDH2* at codon R140. *IDH1* negative cases underwent Sanger sequencing of *IDH2* exon 4.

Results: A total of 6 chondrosarcomas and 17 osteosarcomas yielded DNA of sufficient quality for analysis. No osteosarcomas and 67% of chondrosarcomas (4/6) harbored a somatic mutation in *IDH1*. No chondrosarcomas and two of 17 osteosarcomas showed a somatic *IDH2* mutation. On further pathological review, one of the *IDH2* mutant osteosarcoma cases was actually a dedifferentiated chondrosarcoma with an osteosarcomatous component. The second *IDH2* mutant osteosarcoma case was an unequivocal chondroblastic and osteoblastic osteosarcoma arising in a 12-year-old girl. Of note, the particular *IDH2* mutation (R159C) found in this latter case has not previously been reported and is of unknown biological significance.

Conclusions: *IDH1/2* mutation analysis appears to be a promising biomarker for the distinction of chondrosarcoma from chondroblastic osteosarcoma. Our work is consistent with the previous observation that among mesenchymal tumors, mutations in *IDH1* thus far appear restricted to cartilaginous neoplasms. However our series found one *IDH2* mutation in an osteosarcoma. Analysis of additional cases and testing for accumulation of the distinctive oncometabolite will help further elucidate the significance of these findings.

51 Sampling Modality: Influence on Predicting Outcome in Adult Soft Tissue Sarcomas of the Extremities

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Background: At present, histologic grade is one of the best predictors of outcome in adult soft tissue sarcomas. Core needle biopsies (CNB) have become increasingly popular in the diagnosis and management of these tumours. The objective of this study was to determine if grading of CNB could predict patient outcome.

Design: Seventy-six core biopsies and 65 open biopsies (OB) of spindle cell sarcomas of the extremities were retrieved retrospectively from the archives of our institution. All patients had at least 3 years follow-up. No patient had pre-operative radiation, metastases at the time of presentation, or a diagnosis of liposarcoma of any type. Patient age; tumor type, size, and depth were recorded. All tumours were reviewed histologically and graded using the FNCLCC system and correlated with outcome.

Results: The mean patient age and average tumour size (>5cm) were similar in both groups. However more tumours (50%) were superficial in the OB than the CNB (20%). The range of diagnoses was similar; although more cases of sarcoma NOS were present in the CNB group. The percentages of grades 1, 2 and 3 tumours were similar in both CNB and OB groups. Grade as determined on CNB was not predictive of disease free (Chi^2= 0.747, p=0.688) or overall survival (Chi^2=0.749, p=0.688) by Kaplan Meier analysis. In contrast OB were predictive of disease free survival (Chi^2=8.98, p=0.011) and showed a trend to predicting overall survival (Chi^2=5.37, p=0.068). Interestingly in contrast to OB, grade 3 tumours diagnosed on CNB were not predictive of outcome.

Conclusions: The data suggest that OB is recommended if grading is to be used as a prognostic factor in spindle cell soft tissue sarcomas of the extremities. CNB are likely inadequate for predicting prognosis because of the limited sampling that prevents accurate grading of grade 1 and 2 tumours. It is not clear why tumours classified as grade 3 on CNB have limited ability to predict prognosis. It is possible that our sample number is too small for accurate prediction although this is considered less likely as there was a similar percentage of grade 3 tumours in the OB and the CNB groups. This requires further investigation.

52 Soft Tissue Chordomas: An Analysis of 11 Cases

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Background: The existence of histologically classic chordoma of soft tissue has been questioned as many presumptive cases have been reclassified as parachordoma/myoepithelioma. Utilizing the biomarker brachyury, we have analyzed our experience with 11 cases to validate its existence and define its features.

Design: Departmental and consultation files were searched from 1989-2011 for cases diagnosed as "parachordoma" or "chordoma" arising in soft tissue. Cases were excluded if bone involvement was documented radiologically/surgically, if the patient had a known skeletal chordoma, and/or if the histologic/immunohistochemical features were not typical. Patient age, gender, tumor location and size, and follow up information were noted. Immunostains for brachyury, S100 protein, and cytokeratin were performed.

Results: Eleven of 27 cases met inclusion criteria. There were 9 males and 2 females with ages ranging from 13-71 yrs (mean 44 yrs). Tumors were located on buttock (n=2), wrist (n=2), leg (n=2), toe (n=1), thumb (n=1), ankle (n=1), shoulder (n=1), and chest wall (n=1), ranged in size from 0.5 to 10.9 cm (mean 4.3 cm), and consisted of cords and syncytia of spindled/epithelioid cells with partially vacuolated eosinophilic cytoplasm situated within a myxoid background. Tumors were positive for brachyury (9/9), cytokeratin (10/10), and S100 protein (9/10). Follow up information was obtained in 10 patients with a mean follow up period of 69 months (range 2-212 months). Two patients developed local recurrence (chest wall and leg) and lung metastases, one of whom also developed intrabdominal metastases. A third patient with a buttock primary developed lung metastasis alone. No patient with a distal lesion developed metastasis. Intervals to local recurrence and lung metastasis ranged from 23 to 162 months and 2.5 to 76 months, respectively. One patient died with an unknown disease status; the remainder (n= 7) were alive with no evidence of disease.

Conclusions: 1. Soft tissue chordomas share histologic and immunohistochemical features identical to skeletal chordomas. 2. Distal soft tissue chordomas comprise approximately one half of cases and may have a better prognosis than proximal ones. 3. The existence of soft tissue chordomas implies notochordal remnants are not a prerequisite for chordoma development.

53 *In Vitro* Assessment of Sarcoma Cell Lines Sensitivity to mTOR Inhibitors and Correlation with Genomic Data Evidence Limited Therapeutic Potential

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Background: Sarcomas with complex genetic profiles account for 40% of sarcomas and display highly rearranged genomic profiles. Large series have highlighted high frequency of 10q deletions encompassing *PTEN* tumour suppressor gene locus. *Pten*-deficient murine models develop carcinomas or sarcomas which growth is sustained by AKT-mTOR signaling. In this setting, mTOR inhibitors potently halt tumour growth. Limited therapeutic effects have been evidenced so far in humans but results have rarely been correlated with genomic alterations. We compared *in vitro* effects of therapies targeting mTOR signaling network in sarcoma cell lines and integrated achieved results with genomic data.

Design: We selected seven fully-annotated sarcoma cell lines harbouring either *PTEN* deletion (IB105, IB106, MFH148, LPS78) or not (MH100, MFH152, LPS80). Cell lines were exposed for 96h to either a mTOR inhibitor (rapamycin, RAD001 or PP242), or an anti-IGF-1R antibody (R1507 or R7072) or both. Doxorubicin was used as a positive control. We assessed growth curves by flow cytometry and pro-apoptotic effects by Annexin V-propidium iodide staining. mTOR activity was measured by western blot at basal conditions and under treatment with phospho and native antibodies targeting p70S6K and S6RP, 4E-BP1, PRAS40 and AKT.

Results: Despite potent mTOR inhibition seen on western blot, sarcomas cell lines were poorly sensitive to rapalogs (rapamycin and RAD001) or anti-IGF-1R with antiproliferative effects amounting 37% and 11%, respectively. PP242 elicited strong antiproliferative effects (85% in average) together with apoptosis induction at high doses. Strategies combining mTOR inhibitors to anti-IGF-1R did no elicit additive effects. All *PTEN*-deleted cell lines displayed consistent expression levels of phospho-p70S6K (serine 371) and phospho-S6RP (serine 235). Neither expression levels of mTOR substrates nor PTEN genomic status were predictive of mTOR inhibitors sensitivity. Concurrent genomic alterations proven to affect mTOR signaling were evidenced in all *PTEN*-deleted cell lines including either *AKT1* or *AKT3* deletion.

Conclusions: In our study, only PP242 elicited cytotoxic effects in sarcoma cell lines at high doses. However neither *PTEN* deletion nor mTOR signaling activation were predictive of sensitivity to mTOR inhibitors. Our work provides evidence of limitations to the mTOR addiction model. Based upon genomic data and scientific evidence, we assume that genomic complexity explain these results and jeopardizes mTOR inhibitors's therapeutic potential in sarcomas with complex genetics.

54 The Benign Notochordal Cell Tumor and Ecchordosis Physaliphora Lack the Complex Genomic and Genetic Alterations Commonly Found in the Conventional Chordomas

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Background: Chordoma is a low-grade malignant neoplasm of the axial skeleton, with a histological appearance similar to the notochord present in early embryogenesis. Previous analyses of chordoma using cytogenetics, flouresecent in situ hybridization (FISH), and array comparative genomic hybridization (aCGH) have revealed heterogenous and complex alterations including chromosomal-scale loss and gain, focal gene amplifications, translocations, and chromotripses. Although some of these complex alterations can contribute to the malignant nature of chordoma, the majority of these genomic changes likely represent either late events of tumorigenesis or passenger mutations caused by genomic instability. The early origin of chordoma has remained enigmatic although there is evidence that they arise from a precursor lesion - benign notochordal cell tumor (BNCT). BNCTs share certain cytologic similarities to the conventional chordoma and may be distinguished from the latter morphologically, albeit at times this is very challenging. Another related notochordal lesion is ecchordosis physaliphora (EP). EPs are basilar-pontine lesions which also share histological similarities to the conventional chordoma. But unlike chordoma, they are often discovered incidentally during autopsy, and are thought to be notochordal vestiges remained from embryogenesis. It is currently unknown whether EPs can, in rare instances, develop into chordoma.

Design: Genomic DNA was extracted from formalin-fixed paraffin-embedded specimen of BNCTs and EPs and analyzed using aCGH for copy number changes.

Results: Preliminary aCGH data showed that a benign notochordal cell tumor and an ecchordosis physaliphora lack the complex genomic alteration seen in the majority of conventional chordomas, and instead contain more focal copy number changes at several loci. Furthermore, the aCGH data showed no loss of p16 or PTEN, and no amplification of brachyury, changes commonly detected in chordomas.

Conclusions: Our aCGH results suggest that the deletion of p16 and PTEN loci, the amplification of brachyury gene, and the complex genomic alterations are relatively late events in the pathogenesis of chordoma, and opens the possibility that other unexplored alterations may be important in the early tumorigenesis of chordoma.

55 Clinicopathologic Features of IgG4-Associated Retroperitoneal Fibrosis

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Background: Retroperitoneal fibrosis (RPF) can be divided into idiopathic and secondary cases. Studies have suggested that idiopathic RPF can be classified into cases that are associated with histopathologic features of IgG4-related disease (IgG4-RD) and those that are not. We analyzed previously unclassified cases of RPF to estimate the prevalence of IgG4-RD within this cohort and to compare clinicopathologic features of IgG4-related and non-IgG4-related RPF.

Design: Following chart review, we subclassified 23 RPF cases into idiopathic or secondary categories on the basis of clinical features. Immunohistochemical analysis for IgG4 and IgG was performed on the 22 idiopathic RPF cases with available paraffin blocks. RPF cases were classified as IgG4-related if the IgG4/IgG-positive plasma cell ratio was greater than 40%. Demographic, radiologic, and histologic features were compared between patients with IgG4-related and non-IgG4-related RPF. Statistical analysis was performed using Fisher's exact test or unpaired t-test.

Results: Twelve of 22 (55%) of the idiopathic RPF cases were classified as IgG4-related and 10 (45%) were classified as non-IgG4-related. IgG4-related RPF cases occurred more frequently in men (M:F ratio of 10:2 vs. 4:3), although the difference was not statistically significant. Compared with non-IgG4-related RPF, IgG4-related RPF was more likely to show periaortic involvement (83% vs. 28%, P=0.02), storiform-type fibrosis (83% vs. 28%, P=0.02), and increased eosinophilic infiltration defined as >5 eosinophils per high power field (83% vs. 0%, P<0.001) in association with a plasma cell infiltrate. In the IgG4-related cases, the mean IgG4/IgG ratio was 79%. However, the mean overall number of IgG4-positive plasma cells per high-power field (HPF) was 13, a figure much lower than that generally observed in other organs involved with IgG4-RD.

Conclusions: IgG4-RD accounts for a sizable subset of patients with idiopathic RPF. Both histologic features and immunohistochemical analysis for IgG4 are critical to the diagnosis of IgG4-RD. Findings of plasma cell infiltration, tissue eosinophilia (>5 eosinophils/high power field), and storiform fibrosis suggest the diagnosis of IgG4-RD in RPF. In this context, the IgG4/IgG ratio may be more important in the diagnosis of IgG4-related RPF than the absolute number of IgG4-positive plasma cells/HPF, particularly when extensive fibrosis is the paramount finding.

56 Characterization of Gene Amplification-Driven AMACR Overexpression in Myxofibrosarcoma: Potential Implications in Tumor Progression and Therapeutics

C-F Li, H-Y Huang. Chi-Mei Medical Center, Tainan, Taiwan; Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Taiwan.

Background: Myxofibrosarcoma is genetically complex and remains obscure in molecular determinants of clinical aggressiveness. Despite 5p being frequently amplified in myxofibrosarcoma, the harbored candidate oncogenes remained to be characterized. In our pilot genomic profiling, *AMACR* gene at 5p13.3 was differentially overrepresented in myxofibrosarcomas.

Design: To evaluate clinical significance of *AMACR* gene status and protein expression, we performed immunohistochemical and FISH assays for 105

independent myxofibrosarcomas on tissue microarrays and correlated the results with clincopathological variables, disease-specific survival (DSS), and metastasis-free survival (MFS). *AMACR* mRNA expression folds were by quantified in available fresh samples. *AMACR*-amplified NMFH-1 and NMFH-2 myxofibrosarcoma cell lines were stably silenced with short-hairpin RNAs and evaluated by BrudU and soft agar assays.
Results: Present in 28/105 (27%) primary myxofibrosarcomas, AMACR overexpression was highly associated with higher grades ($p<0.001$) and gene amplification ($p<0.001$; 17/28, 61%). However, *AMACR* gene was non-amplified in 11/28 (39%) tumors overexpressing AMACR. Gene amplification was positively related to higher grades ($p=0.002$) and advanced stages ($p=0.039$). Besides higher grades, both AMACR overexpression ($p<0.0001$ for both endpoints) and gene amplification (DSS, $p=0.0002$; MFS, $p=0.0062$) univariately correlated with poor prognosis. However, only AMACR overexpression independently portended adverse outcomes (DSS, $p=0.0071$; MFS, $p=0.0007$). In vitro, stable AMACR-knockdown NMFH-1 and NMFH-2 lines showed suppressed cell proliferation and anchorage-independent growth of myxofibrosarcoma cells.
Conclusions: Driven mostly by gene amplification, AMACR overexpression is present in a significant subset and confers tumor aggressiveness in myxofibrosarcomas.

57 Immunohistochemical Profile of 494 Genetically-Confirmed Ewing's Sarcoma Cases

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Background: Ewing's sarcoma family of tumors (ESFT) represents a well established clinical, morphological and molecular entity. The aim of the present study is to describe the complete immunohistochemical profile in a large series of genetically-confirmed ESFT.
Design: A total of 494 genetically-confirmed ESFT were included in the study. The tumors were divided according to the histological subtypes into conventional, PNET and atypical variants. Immunohistochemical analysis was performed on tissue microarray sections using antibodies to determine mesenchymal/neural (CD99,Fli1,HNK-1,Cav-1,S100), epithelial (pan-CK, CK20,EMA,CEA), neuroendocrine (synaptophysin, chromogranin-A, NF) and/or myogenic differentiation (actin, desmin, myogenin). A correlation between IHC expression and histological subtypes was achieved.
Results: CD99, HNK-1, CAV-1 and Fli1 were the most positively expressed antibodies in the present series. Epithelial differentiation (pan-CK) was observed in 19.2% of tumors, mainly in the atypical variant. CK20 was absent in all cases, however EMA and CEA were seen in 6.6% and 20.8% of ESFT respectively, and also predominantly in the atypical subtype. ESFT did not reveal desmin or myogenin. A low proportion of neuroendocrine markers were expressed.
Conclusions: The accurate diagnosis of ESFT has been supported by genetic analysis and immunohistochemical profiling, mainly represented by positivity to CD99, Fli1, HNK-1 and CAV1. The epithelial expression including EMA and/or CEA positivity in small round cell tumor does not exclude the diagnosis of ESFT. Presence of a myogenic differentiation should rule out the diagnosis of this entity. The atypical ESFT present immunohistochemical features that overlap with other small round cell tumors, thus molecular studies should be used in order to attain an accurate diagnosis.
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58 Angiofibroma of Soft Tissue: Clinicopathologic Characterization of a Distinctive Benign Fibrovascular Neoplasm in a Series of 37 Cases

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Background: Fibroblastic tumors with prominent vascularization are rare, mainly limited to nasopharyngeal angiofibroma and cellular angiofibroma. We have become aware of a distinct benign soft tissue neoplasm with fibroblastic cytomorphology and a very noticeable vascular pattern which may be mistaken for a low grade sarcoma.
Design: Thirty-seven cases of a distinctive benign fibrovascular soft tissue tumor identified between 2000 and 2011 were retrieved from consultation files. Clinicopathologic and follow-up information was obtained from referring pathologists. H&Es were reviewed. IHC stains for CD34, EMA, SMA, desmin and S100 were performed.
Results: Patients were 25 women and 12 males, aged 6-86 years old (median 49 y). The tumors presented most commonly as a slowly growing painless mass in the soft tissues of the extremities, mainly the lower extremity, often in relationship to joints or fibrotendinous structures. Most lesions (29) were well circumscribed, 1.2-12 cm in size (median 3.5 cm). Microscopically, they were characterized by 2 components: a uniform proliferation of bland, spindle-shaped cells with inconspicuous cytoplasm and ovoid nuclei set in a variably myxoid stroma, and a prominent vascular network with numerous small branching thin-walled blood vessels, often accompanied by medium-sized round or ectatic vessels. Mitoses (1-4/10 hpf) were occasionally present (9 cases). Mild degenerative nuclear atypia was uncommon (5 cases). Tumor cells expressed EMA (usually focally) in 16/36 cases (44%), CD34 and SMA in 5 (14%) and desmin in 4 (11%); none expressed S100 protein. Cytogenetically, 5 out of 6 cases analyzed showed simple karyotypes with a balanced t(5;8) translocation. All patients were treated by surgical excision. Follow-up information was available for 28 patients (range 6-144 months; mean 51.9 months). Most patients showed no evidence of disease, regardless of the status of surgical resection margins. Four patients developed local recurrence 9, 13, 36 and 120 months after the primary tumor was removed; one developed a second recurrence 2 months later. None of the patients developed metastasis.
Conclusions: Angiofibroma of soft tissue is a previously unrecognized fibrovascular soft tissue tumor with distinctive morphology, arising most commonly in the extremities of middle aged adults. Preliminary data suggest that these tumors have a distinct and reproducible karyotype. The clinical course is benign, with rare local recurrences and no evident metastatic potential. Simple local excision seems to be adequate treatment.

59 Interphase Fluorescent In Situ Hybridization Patterns in Translocation Associated Sarcomas

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Background: Interphase fluorescent in-situ hybridization (FISH) using break-apart probes is commonly used to diagnose chromosomal rearrangements in translocation associated sarcomas and is routinely performed on formalin fixed paraffin embedded tissue (FFPE). In this technique, the separation of the two probes indicate rearrangement of one of the genes without identification of the partner gene. It is a robust technique with clear signals of one fused signal of the probes representing the normal gene and two split signals representing the rearranged gene. This standard rearrangement pattern (SRG) can sometimes be accompanied by additional complex FISH patterns (CFP). These include duplications or amplification of normal gene and rearranged gene, loss or gain of 5' or 3' sequences and combinations of the above. In this study we retrospectively analyzed the FISH patterns in various translocation associated sarcomas.
Design: We collected pathological data and FISH results on the following translocation associated sarcomas for the last 5 years from the database of the Pathology Department at Memorial Sloan-Kettering Cancer Center. FISH results were available on twenty-five (25) Synovial Sarcomas (SS), eleven (11) Alveolar Rhabdomyosarcomas (ARMS), nine (9) desmoplastic small round cell tumors (DSRCT), four (4) Low grade fibromyxoid sarcomas (LGFMS), and three (3) myxoid liposarcomas (MLS).
Results: In this group complex FISH patterns (CFP) were encountered in SS, ARMS and myxoid liposarcomas. DSRCT and LGFMS showed only SRG of *EWSR1* and *FUS* respectively. Eight out of twenty-five (32%) of Synovial sarcomas displayed complex FISH patterns, which included SRG and duplication or additional copies of native *SYT/SS18* in 6 cases with or without loss of 5 ' or 3' sequences. ALL ARMS except for one case (90%) had complex FISH pattern. Duplication or multiple copies of SRG of the *FKHR*/*FOXO1* gene was the most common complex pattern. Of the three MLS, two showed CFP which included duplication and loss of 5' in addition to SRG of the *CHOP/DDIT3* gene. Both cases were high grade.
Conclusions: 1) Complex interphase FISH patterns are not uncommon in translocation associated sarcomas
2) In these small series, CFPs are highly prevalent in alveolar rhabdomyosarcoma
3) It is interesting that desmoplastic small round cell tumor, a highly aggressive neoplasm showed only standard rearrangement of the EWSR1 gene in all cases.

60 *TFE3* Gene Rearrangement Status Is Heterogeneous in Alveolar Soft Part Sarcomas: A Study by Dual-Color Chromogenic In Situ Hybridization on Formalin-Fixed Paraffin-Embedded Samples

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Background: Alveolar Soft Part Sarcoma (ASPS) is a rare soft tissue sarcoma of unknown histogenesis. Cytogenetically, ASPS invariably shows a specific unbalanced translocation involving the chromosomes X and 17, which results in *ASPL-TFE3* fusion gene. Using dual-color chromogenic in situ hybridization (dc-CISH) that enables simultaneous morphologic and cytogenetic assessment at cellular level under bright-field microscope, we examined cell-type-specific *TFE3* gene status on formalin-fixed paraffin-embedded samples (FFPEs).
Design: We retrieved 5 ASPS cases. Prior to dc-CISH, they were confirmed by RT-PCR to harbor *ASPL-TFE3* fusion. The FFPEs of these were studied by dc-CISH using custom-made break-apart probes for *TFE3*, visualized by DAKO duoCISH. The *TFE3* gene status of neoplastic and non-neoplastic cells was separately evaluated by examining 100 cells each. The dc-CISH signal pattern of typical unbalanced translocation shows an additional isolated 3' signal. TFE3 protein expression was also evaluated immunohistochemically by assessing 1000 cells of each cell type.
Results: ASPS tumor cells showed heterogeneous signal pattern by dc-CISH in all samples. The cells with typical unbalanced translocation pattern ranged from 1 to 19% (mean 13%) of tumor cellularity, while the cells with split 3'- and 5'- signals and thoese with no translocation patterns constituted 8-70% (mean 28%) and 16-65% (mean 36%), respectively. *TFE3* rearrangement was absent in non-neoplastic cells. Immunohistochemically, diffuse and strong nuclear TFE3 staining was observed in 86.3-96.5% (mean 92.7%) of tumor cells, whereas non-neoplastic cells showed no immunoreactivity.
Conclusions: The *TFE3* gene status was found quite heterogeneous in ASPS and only a subset of tumor cells showed the signal pattern of typical unbalanced translocation. This intratumoral genetic heterogeneity has never been documented by conventional RT-PCR and FISH, and is in contrast to what one would expect for typical translocation-associated sarcomas. Thus, care should be taken when diagnosing ASPS by CISH or FISH. Despite cytogenetic heterogeneity, TFE3 protein expression was diffuse and homogeneous. Such discordance may indicate rearrangement-independent expression mechanisms, and examples may include complex epigenetic regulation.

61 Evaluation of the Mitogen-Activated Protein Kinase Pathway in Osteosarcoma

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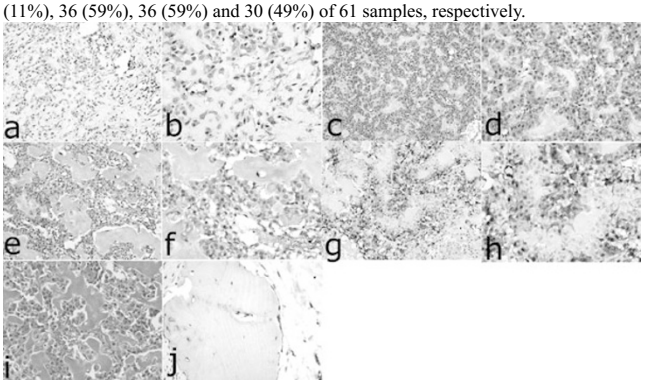
Background: The mitogen-activated protein kinase (MAPK) pathway has been implicated in the progression of osteosarcoma (OS). Recent studies have demonstrated

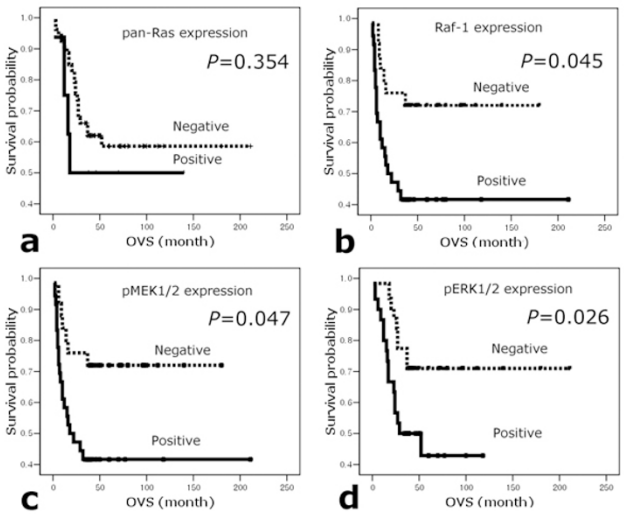
that inhibitors of MAPK pathway components have antitumor activity in preclinical models of OS. However, less is known about the prognostic value of components of the MAPK pathway.

Design: We evaluated the expression of pan-Ras, Raf-1, pMEK1/2 and pERK1/2 in 61 patients with primary localized OS and assessed their prognostic influence on event-free survival (EFS) and overall survival (OVS) using immunohistochemistry.

Results: The markers pan-Ras, Raf-1, pMEK1/2 and pERK1/2 were expressed in 7 (11%), 36 (59%), 36 (59%) and 30 (49%) of 61 samples, respectively.



Patients whose tumors expressed Raf-1 or pMEK1/2 had poorer EFS and OVS than patients whose tumors showed negative staining for Raf-1 (P=0.016 and P=0.045, respectively) or pMEK1/2 (P=0.017 and P=0.047, respectively). Patients whose tumors stained positive for pERK1/2 had poorer OVS than the group whose tumors did not express pERK1/2 (P=0.026).



Notably, positive pMEK1/2 expression was predictive of shorter EFS and OVS (P=0.024 and P=0.042, respectively).

Conclusions: Our study provides further evidence that activation of the MAPK pathway plays a role in the aggressive behavior of OS. In addition, our study suggests that further investigation of MEK inhibitors in OS is warranted.

62 Intimal Sarcoma Represents the Most Common Primary Cardiac Sarcoma: A French National Retrospective Clinicopathologic and Molecular Study of 84 Cardiac Sarcomas. Implications for Diagnosis and Treatment

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Background: Sarcomas constitute the majority of malignant primary cardiac tumors. Angiosarcomas are predominantly right-sided and other sarcomas (undifferentiated, leiomyosarcomas…) left-sided, whereas intimal sarcomas involve large vessels. We report novel molecular and pathologic features within the largest described series of primary cardiac sarcomas.

Design: 84 cases of sarcomas involving the heart were retrieved from the French national territory, between 1977 and 2010. Histological materials were centralized and reviewed. Complementary immunohistochemistry, FISH, qPCR, arrayCGH, RT-PCR were performed on paraffin embedded material or frozen sample if available.

Results: There were 56 women and 28 men, 48 years mean age (range 17-77). Right and left heart were respectively involved in 37 and 47 cases. There were 36 intimal sarcomas, 20 angiosarcomas, 18 unclassified sarcomas, 7 synovial sarcomas, 2 leiomyosarcomas and 1 PNET. All 20 angiosarcomas originated from the right heart, whereas 30 of the intimal sarcomas and 13 of the unclassified sarcomas were from the left heart. Before complementary techniques, intimal sarcomas were diagnosed as unclassified sarcomas, leiomyosarcomas, rhabdomyosarcomas, myxofibrosarcomas... MDM2 immunohistochemical overexpression was observed in all intimal sarcomas with *MDM2* amplification, but also in 8/18 unclassified sarcomas without *MDM2* amplification. Rhabdomyosarcomas were all intimal sarcomas. arrayCGH shows a complex genomic profile, with 12q13-14 amplicon, 4q12 amplicon and 9p21.3-p21.2 deletion.

Conclusions: Intimal Sarcoma involves the heart and appears in our series as the most common primary cardiac sarcoma, predominantly left-sided. MDM2 immunohistochemical overexpression is not sufficient to make this diagnosis. Molecular aberration should be proved. As resections are limited in left atrium, this histological subtype could benefit from therapies that target *PDGFRA* or *MDM2*.

63 *KRAS* Mutation in Lipomas, Atypical Lipomatous Tumors/Well-Differentiated Liposarcomas (ALT) and Dedifferentiated Liposarcomas (DDLS)

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Background: Mutations in members of the RAS family of oncogenes (*KRAS, HRAS,* and *NRAS*) occur in many cancers. *HRAS* mutations have been studied in well-differentiated liposarcoma and DDLS. However, *KRAS* mutation status is not well-studied in lipomatous tumor and reports are few, if any. Herein, we report *KRAS* mutation status in lipoma, ALT and DDLS cases diagnosed in our institution.

Design: We searched the pathology archives of our hospital during 2002 to 2011 and randomly selected 21 cases of lipoma, 9 cases of ALT and 6 cases of DDLS to examine *KRAS* mutation status. Areas of tumor were identified and microdissected from formalin-fixed, paraffin-embedded tissue in all cases. Two different areas, one with ALT-like and one with poorly differentiated sarcomatous features, were selected in one case of DDLS to compare mutational status. *KRAS* mutation on codons 12 & 13 was tested by peptide nucleic acid (PNA)-clamp quantitative PCR and reflexed to direct Sanger sequencing when PCR was positive.

Results: The median ages of patients are 49, 67 and 76.5 years old with lipoma, ALT and DDLS, respectively. There is no gender predilection in all groups. The location of lipoma and DDLS was diverse, but ALT shows predilection for thigh (66%, 6/9). Out of 21 cases of lipoma, one case (4.8%) showed Gly12Val (GGT>GTT) *KRAS* mutation. In ALT group, one case (11.1%) showed Gly12Cys (GGT>TGT) *KRAS* mutation. *KRAS* mutation was not found in DDLS.

Conclusions: Although the number of cases in this study is low, activating *KRAS* mutations involving codons 12 & 13 appear to be rare in lipomatous tumors. This result contrasts with a report of frequent (21%, 4/19) *HRAS* mutations in DDLS. A comprehensive analysis of lipomatous tumors including global genetics and/or epigenetic approaches targeting a variety of oncogenic pathways (e.g. RAS, JAK, PIK3CA, or WNT) is needed to reveal the primary oncogenic trigger.

64 Mesenteric and Superficial Fibromatosis Are Distinctly Different Tumors. A Proteomic Analysis Using Laser Microdissection and Mass Spectrophotometry

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Background: Mesenteric fibromatosis (a variant of deep fibromatosis) are locally aggressive clonal fibroblastic proliferations due to dysregulation of the Wnt/beta-catenin signalling pathway. Superficial fibromatosis exhibits striking histomorphologic and immunophenotypic overlap but genetically lack beta-catenin and APC mutations that are frequently seen in mesenteric fibromatosis. The biology of these tumors are also different. In this study, using archival formalin fixed paraffin embedded (FFPE) material, laser microdissection (LCM) and mass spectrophotometry were performed and the proteomics of these two disparate lesions in tandem with normal dermal collagen and scar tissue were evaluated.

Design: FFPE Archival material of superficial fibromatosis (SF, n=4), mesenteric fibromatosis (MF, n=4) Scar (n=4) and normal dermal collagen (DC, n=4) were retrieved from the OSU tissue archives. The diagnoses were validated and areas of interest were marked as a guide for LCM. Laser capture was performed using a Zeiss Palm Microbeam on 10um sections of tissues. The microdissected tissues were then digested with trypsin and analyzed using a high-resolution LTQ-Orbitrap tandem mass spectrometer. Data were analyzed using Matrix Science Mascot database search software.

Results: Comparison of the SF and MF revealed unique protein expression differences: Complement C3, C4 and Immunoglobins were upregulated in the MF compared to SF, indicating a possible immune mechanism. Tenascin and Collagen alpha-1were upregulated in SF. Ingenuity Pathway analysis of the data showed depletion in protein involved in Actin cytoskeletal signaling in the MF. The SF showed enrichment of proteins associated with acute phase response and ILK signaling. LMPC proteomics of scar vs normal DC showed significant differences in protein expression. Many more protein changes were seen in the SF and MF vs. normal comparisons. For MF, we observed downregulation of collagen alpha-1. Upregulated proteins included Myosin 10, Cytoskeleton-associated protein 4, complement C3, Serpin H1 and Perostin. SF vs DC showed downregulation of Decorin, alpha-2_macroglobulin and Prolargin. Proteins that were upregulated included Perostin and Tenascin.

Conclusions: Our data shows distinct clustering of differentially expressed proteins in MF and SF, supporting their molecular genetic differences. The differentially expressed proteins require further evaluation and validation as potential biomarkers and/or therapeutic targets.

65 Clinical Utility of MYH9/USP6 Fusion Transcript Detection and USP6 Expression in Nodular Fasciitis

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Background: Nodular fasciitis (NF) is a self-limited myofibroblastic lesion which can be misdiagnosed as a sarcoma as a result of its rapid growth, prominent mitotic activity, and cellularity. Recently, a recurrent translocation t(17;22) has been identified, resulting in the fusion of *USP6* to the promoter region of *MYH9*, and driving increased USP6 expression. We developed a novel RT-PCR assay to detect MYH9/USP6 fusion transcripts in formalin fixed paraffin embedded (FFPE) and frozen tissue, and examined USP6 protein overexpression by immunohistochemical (IHC) study.

Design: 11 cases of NF with available clinical information were retrieved from the pathology files of our institutions from 2004-2011. Ages of presentation ranged from 12 to 65 years with a male: female ratio of 2.7:1. Locations included the upper extremity, face, and pelvis. Novel PCR primers were designed to optimize an RT-PCR assay for the detection of the two previously described splice variants using frozen and FFPE specimens. Representative FFPE tissue from 11 NF cases and 1 case of cranial fasciitis, and frozen tissue from 1 NF case, were tested by RT-PCR. Amplicons from all positive cases were characterized by sequencing. Corresponding immunohistochemical analysis for USP6 expression (polyclonal, 1:20, Sigma-Aldrich) was also performed in all cases.

Results: 9 of 11 (81%) NF cases were positive for MYH9/USP6 fusion transcripts by RT-PCR while the cranial fasciitis specimen was negative. All of the positive cases showed increased expression of USP6 by IHC.

Conclusions: RT-PCR for MYH9/USP6 fusion transcript detection is diagnostically useful for NF surgical specimens and core needle biopsies. In our study, the presence of the fusion transcript correlated with increased expression of USP6, as detected by IHC. RT-PCR and IHC testing of additional cases is ongoing, and the results are being validated using a *USP6* break-apart FISH assay currently under development.

66 Muc-4 Expression and FUS Rearrangement in Sclerosing Epithelioid Fibrosarcomas: A Pathological Study of 20 Cases Further Supporting Relationship with Low Grade Fibromyxoid Sarcoma

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Background: Sclerosing epithelioid fibrosarcoma (SEF) is an aggressive sarcoma with a predilection for the deep soft tissues of the extremities and trunk and a propensity for local recurrence, metastasis and tumor-related death. A subset of SEF shows morphologic and molecular overlap with low grade fibromyxoid sarcomas (LGFMS). LGFMS characteristically harbors FUS-CREB3L1/L2 and has recently been shown to express Muc-4, which can be used as a diagnostic immunohistochemical marker. We have studied MUC-4 expression and evidence of FUS rearrangement in a large series of SEF. Pathological findings and clinical outcome were compared to a cohort of 14 "classic" LGFMS.

Design: 20 cases of SEF and 14 LGFMS were retrieved, reviewed and stained for Muc-4. RT-PCR and/or FISH for FUS-break apart were performed. Clinical follow up was obtained.

Results: 14/20 SEF and 14/14 LGFMS showed Muc-4 expression; FUS-rearrangement was detected in 2/9 SEF (both Muc-4 positive) and 8/8 LGMFS tested. Clinical follow up was obtained from 19 SEF cases (4 to 216, median 31 months) and 13 LGFMS (2 to 121, median 37 months). Metastastic disease occurred in 14/19 SEF patients (metastasis-free survival 0 to 120, median 12 months) and 6 patients died of tumor (survival 4 to 73, median 31.5 months). There was no significant difference between Muc-4 positive and Muc-4 negative SEF with regard to metastasis and overall survival. Of 13 LGFMS cases, 3 had metastasized (2 at presentation, one after 53 months), all alive 2, 7 and 121 months after diagnosis. Metastasis-free survival and overall survival of Muc-4 positive SEF were significantly shorter when compared to LGFMS.

Conclusions: A high proportion of SEF expresses Muc-4 and a smaller proportion shows FUS rearrangement. The findings support a histogenic relationship between LGFMS and SEF. The fact that SEF shows a more aggressive biological behavior in respect to metastatic potential and survival justifies the continuous distinction between the entities.

67 Dedifferentiated Liposarcoma of the Extremities: Relative Incidence Compared with Atypical Lipomatous Tumors of the Extremities, and Clinicopathologic Features Including Two Cases with Morphology of So-Called "Inflammatory MFH"

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Background: Dedifferentiated liposarcoma (DDLPS) is reportedly rare in the extremities compared with retroperitoneum. Advances in identification through IHC for the proteins, CDK4 and MDM2, may increase recognition of these tumors when occuring in the extremities exclusively as sarcoma, NOS. Accurate diagnoses is crucial because of improved prognosis compared with sarcoma, NOS.

Design: The Pathology database was searched for non-retroperitoneal ALT and liposarcomas for 2001 to 2011. Clinical, radiologic, and pathologic features were evaluated. Radiology and pathology reports were reviewed, and scans and slides were re-examined. The relative frequency of DDLPS was compared with ALT.

Results: Table One lists pure ALT, mixed ALT/DDLPS and pure DDLPS.

Non-Retroperitoneal DDLPS and ALT

Feature	PURE ALT	MIXED ALT- DDLPS	PURE DDLPS
AVERAGE AGE	62.6 years	70 years	61 years
GENDER	18 F, 20 M	2 F, 6 M	2 F, 6 M
AVERAGE SIZE	18.3	12.4	10
LOCATION	30 E, 5 T, 2 each H&N and G	8 E	6 E, 2 T

F = female, M = male, Size is in centimeters E= Extremities, T= Trunk, G= Groin, H&N= head and neck

Thirty percent of non-retroperitoneal liposarcomas consisted of dedifferentiated LPS eight of which were mixed ALT/DDLPS and eight pure DDLPS. Only one patient progressed from ALT to DDLPS. Dedifferentiated components included two cases of "inflammatory MFH", one case with myofibroblastic differentiation, one case mimicking sclerosing epithelioid fibrosarcoma, two cases simulating adult type fibrosarcoma, three cases with the appearance of an epithelioid sarcoma (INI-1 intact), and the remaining dedifferentiated components consisted of high grade pleomorphic undifferentiated sarcoma (n = 7). There was no difference in patient age or tumor size. Males outnumbered females by 3:1 for dedifferentation. Radiology supported low grade fatty tumor for the pure ALT or predominantly pure ALT, and suggested DDLPS for a few of the mixed DDLPS cases, while the pure DDLPS cases were interpreted as suspicious for high grade sarcoma. For pure DDLPS, CDK4, MDM2 and/or karyotypes supported the diagnosis.

Conclusions: Dedifferentiated liposarcoma may become increasingly recognized as in non-visceral soft tissues due to CDK4 and MDM2 immunohistochemistry. The range of morphologies in the extremities includes the "inflammatory MFH" like pattern noted in retroperitoneal DDLPS.

68 Role of Macrophages and Tumor Angiogenesis in Desmoid-Type Fibromatosis

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Background: Desmoid-type fibromatosis (DTF) is a rare soft tissue tumor with fibroblastic features affecting 2 to 4 individuals per million population per year. Despite its bland microscopic appearance, the tumor behaves aggressively. Although unable to metastasize, DTF tends to recur and local recurrences in anatomically critical sites can be fatal. Tumor associated macrophages (TAM) play an important role in tumor development through the activation of angiogenesis, particularly in cases of epithelial malignancies. The aim of this study is to investigate the prognostic significance of TAMs and the number of microvessels in DTF.

Design: Tumor macrophages (CD163), microvessel density (CD31), and beta-catenin were investigated on 69 primary DTF cases with follow-up information. *CTNNB1* mutations were also studied.

Results: High density of tumor macrophages and high number of microvessels were associated with a significantly worse recurrence-free survival ($P = 0.03$ and $P = 0.007$ respectively). There was a significant correlation between microvessel density and CD163-macrophages ($P=0.02$). Furthermore, combination of high number of tumor macrophages and high microvessel density (MVD) greatly improved the statistical significance ($P = 0.000005$).

Conclusions: Macrophages and microvessels may play an important role in the biologic behavior of DTF. This finding could help in the clinical management of patients with DTF.

69 Therapeutic Accuracy and Diagnostic Utility for Open Biopsy, Core Needle Biopsy and Fine-Needle Aspiration in a Series of 282 Biopsy Procedures: Comparison with Resection Diagnoses

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Background: Interpretation of musculoskeletal biopsies is challenging with reported diagnostic accuracy for open (OB) and core needle (CNB) biopsies being as low as 82%. Diagnostic accuracies of 76-98% and 74-99% have been reported for CNB and fine-needle aspiration (FNA) respectively. Most studies of CNB and FNA have reported accuracy for the separation of benign from malignant lesions. This does not represent true therapeutic accuracy as therapy for malignancies often depends on specific histologic type. Clinical utility is lower than therapeutic accuracy because some FNA and CNB are followed by confirmatory open biopsy. This diminishes the clinical utility of the initial FNA or CNB.

Design: We compared the therapeutic accuracy and clinical utility of OB, CNB and FNA in a series of 282 patients (121 OB, 132 CNB, 65 FNA) who had subsequent definitive therapeutic resections at a single medical center. Biopsy results were categorized as therapeutically correct, minor error, major error, insufficient or confirmation by open biopsy needed. We also calculated the therapeutic accuracy and clinical utility for the combination of CNB and FNA when both were performed at the same time.

Results: Therapeutic accuracy was 86% for OB, 81% for CNB and 64% for FNA. Therapeutic accuracy for the combination of CNB and FNA was 80%. Clinical utility was 86% for OB, 76% for CNB and 38% for FNA. The clinical utility for the combination of CNB and FNA was 75%. Percentage of biopsies insufficient were 0%, 17%, 15% and 0% for OB, CNB, FNA and combined FNA and CNB respectively.

Conclusions: The reduced clinical utility of CNB and FNA was due to confirmatory open biopsies performed before definitive therapy in some cases. FNAs clinical utility was significantly less than that of CNB because of clinician's discomfort with purely cytologic diagnoses. The therapeutic accuracy of FNA was closer to that of CNB (64% vs. 81%) than was its clinical utility (38% vs. 76%). Therapeutic accuracy and clinical utility were not improved by combining CNB and FNA when compared to CNB alone.

70 Rearrangement of *DDIT3 (CHOP)* in Perivascular Epithelioid Tumors (PEComas): A Novel Finding

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Background: The genetic events underlying the development of soft tissue perivascular epithelioid tumors (PEComas) have not been fully elucidated. A prior comparative genomic hybridization study has shown genetic gains and losses on several chromosomes including gains involving X, 12q, 3q, 5 and 2q. Loss of expression of the tumor suppressor genes *TSC1* and *TSC2* has also been shown in some PEComas. Prompted by a recent case of PEComa found during routine pathological evaluation to have rearrangement of *DDIT3*, we evaluated a larger series of well-characterized PEComas of soft tissue, gynecologic and cutaneous origin for evidence of *DDIT3* rearrangement.

Design: Inclusive of the index case, 13 morphologically and immunophenotypically classical PEComas were retrieved from our institutional and consultation archives. In each case, H&E stained slides were reviewed and tumor areas marked. Three corresponding unstained slides were submitted for FISH analysis using the Vysis LSI *DDIT3* dual color break apart probe. Approximately 60 cells were evaluated in each case and the cutoff value for positivity was 15.5%.

Results: The tumors occurred in 4 male and 9 female patients, ranging in age from 28 to 72 years (mean 49 years). Tumors involved soft tissue/viscera (N=7), skin (N=4), and uterus (N=2). Cases were classified as histologically benign (N=8), of uncertain malignant potential (N=1) and histologically malignant (N=4). Rearrangements of the *DDIT3* locus were identified in 3/13 cases (23%), all of soft tissue origin (1 malignant, 1 uncertain, 1 benign).

Conclusions: This represents, to the best of our knowledge, the first report of *DDIT3* rearrangements in PEComas. This finding suggests the presence of a *DDIT3*-containing translocation in at least a subset of PEComas, although the fusion partner(s) is (are) currently unknown. It is unlikely that *DDIT3* rearrangement will prove to be related to clinical behavior in PEComas, given its presence in tumors both with and without morphological features of malignancy. *DDIT3* is known to be involved in adipocyte differentiation, cell cycle progression and malignant transformation, and may prove to be important in the pathogenesis of PEComas.

71 Global MicroRNA Expression of Sarcomas. A Study of Formalin Fixed Paraffin Embedded Tissues Using NanoString MiRNA Assay PlatForm

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Background: Sarcomas are malignant heterogenous tumors of mesenchymal derivation. The pathogenesis of many of the different histologic sub-types remains poorly understood. microRNAs (miRNAs) are endogenous non-coding single stranded RNA's of 18-25 nucleotides in length that act as expression modulators of genes involved in fundamental cell processes and its dysregulation have been implicated in cancers.

In this study we used a selective miRNA screening platform to study the comparative global miRNA expression signatures in a cohort of human sarcomas with the patients own normal tissues serving as controls from formalin fixed paraffin embedded tissue (FFPE) samples.

Design: FFPE blocks of 20 sarcomas from 4 histologic types were retrieved including: myxoid/round cell liposarcoma (n=4); well differentiated liposarcoma (WDLPS, n=4), dedifferentiated liposarcoma (n=4) and synovial sarcoma (SS, n=4). All the cases were re-reviewed and the diagnosis was further validated by additional molecular testing where appropriate. The areas of tumor and uninvolved (or normal appearing areas) were identified and marked. 19 adjacent normal tissues from the same patients were available and multiple 1.75mm cores were obtained from both tumor and the uninvolved normal tissues. Total RNA for NanoString nCounter Human miRNA expression analysis was isolated from FFPE human sarcomas (n=20) and subjacent normal tissue (n=19) using RecoverAll Total Nucleic Acid isolation Kit for FFPE tissues (Ambion, Inc) according to manufacturer protocol and analyzed using NanoString technology (NanoString, Seattle, Washington). The results were processed using SAM and cluster analysis module.

Results: Clustering analysis shows a distinct global difference in expression patterns between the normal and sarcoma tissues. The different sarcoma subtypes reveal expression signatures in an unsupervised hierarchical clustering analysis. Of note, more miRNAs were down-regulated than upregulated. MiR-143 showed significant down-regulation in WDLPS and SS while miR-145 showed down-regulation only in WDLPS.

Conclusions: Our data indicate distinctly different patterns of miRNA expression in normal and sarcoma samples regardless of histologic subtype. The unique expression miRNA signatures for different sarcoma subtypes may have diagnostic, prognostic or even therapeutic implications.

72 Well-Differentiated and Dedifferentiated Liposarcomas with Prominent Myxoid Stroma: Analysis of 55 Cases

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Background: Well-differentiated (WD) liposarcoma (LPS) has significant recurrent potential but does not metastasize; dedifferentiated (DD) LPS has a 15-20% metastatic risk. WD/DDLPS contain ring and giant marker chromosomes with amplified material from 12q13-15, resulting in overexpression of MDM2 and CDK4. Occasional cases of WD/DDLPS show prominent myxoid stroma, leading to potential confusion with other sarcoma types. Correct diagnosis is crucial, owing to marked differences in clinical behavior. We analyzed the clinicopathologic, immunohistochemical, and genetic features of WD/DDLPS with myxoid stroma.

Design: 55 LPS cases (22 WD; 33 DD) with prominent myxoid stroma were retrieved from consultation and surgical files (M/F 34/21; median age 55 yr; range 14-82 yr). Immunohistochemistry (IHC) for MDM2 and CDK4 was performed; cytogenetic findings were recorded. Clinical follow-up was obtained.

Results: Most tumors (78%) arose in the retroperitoneum or abdomen. Tumor size ranged from 2-50 cm (mean 17 cm). All tumors had a conventional adipocytic WDLPS component. In 15 WD cases, the myxoid component contained myxoid LPS-like plexiform vessels; the degree of nuclear atypia and pleomorphism distinguished such areas from myxoid LPS. Other cases showed a hypocellular myxoid spindle cell appearance with admixed adipocytes. The DDLPS cases showed a broader range of patterns in the myxoid areas. In 16 cases, myxofibrosarcoma-like curvilinear vessels were prominent. In other cases, the myxoid component ranged from relatively bland myxoid LPS-like morphology to a higher grade pleomorphic spindle cell appearance. By IHC, all 37 cases evaluated were positive for MDM2; CDK4 was positive in 64%. Cytogenetic findings in 8 cases showed ring and giant marker chromosomes. Follow-up in 30 cases (9 WD; 21 DD) ranged from 8 mo to 20 yr (mean 7 yr). All patients except 1 had local recurrences (up to 4). Only 1 patient so far with DD had distant metastases to lung. At last follow-up, 11 patients died of disease (10 with DD; 1 with WD), 1-20 yr after diagnosis.

Conclusions: WD and DDLPS with prominent myxoid stroma can resemble other sarcoma types, especially myxoid LPS and myxofibrosarcoma. Clinical presentation (large retroperitoneal or abdominopelvic tumor) is a clue to the correct diagnosis; the degree of nuclear atypia and pleomorphism excludes myxoid LPS. Thorough sampling is needed to identify a conventional WD component. In the absence of such a component, IHC for MDM2 and CDK4 and genetics can be useful to confirm the diagnosis.

73 ATF2 in Synovial Sarcoma

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Background: The SS18-SSX fusion oncoprotein in synovial sarcoma has no DNA binding domain and its mechanism of transcriptional dysregulation has been unclear. Using mass spectrometry, reciprocal immunoprecipitation (IP), siRNA and chromatin IP studies in synovial sarcoma cell lines, we were able to identify Activating Transcription Factor 2 as its probable DNA binding partner, abnormally bridged to the TLE1 corepressor by SS18-SSX. We sought to extend this study to other model systems and patient specimens, characterize target genes and survey ATF2 expression in sarcomas.

Design: Models: 2 synovial sarcoma cell lines, HEK293 cells engineered to express tagged SS18 or SS18SSX, mouse model synovial sarcoma cells. Patient specimens: 11 frozen primary synovial sarcomas including SSX1 & 2 variants, and tissue microarrays representing 57 synovial sarcomas and 219 other tumors. Methods: reciprocal IP, glycerol gradient fractionation, cell viability & apoptosis assays, expression profile analysis, chromatin IP and immunohistochemistry on tissue arrays

Results: ATF2, SS18-SSX and TLE1 reciprocally immunoprecipitate in patient specimens (SSX1 and SSX2), as do their murine homologues in mouse synovial sarcomas generated by conditional expression of SS18-SSX2. ATF2 binds SS18 but cofractionates with the repressor TLE1 only in the presence of SS18-SSX. Both ATF2 and TLE1 knockdowns induce apoptosis in synovial sarcoma. Seven target genes of ATF2, bearing its CRE consensus binding sequence in their promoters, were identified and all appear to be abnormally repressed in patient synovial sarcoma specimens. Chromatin IP assay demonstrates ATF2, SS18-SSX and TLE are all resident at the CRE elements in these target genes, true also of murine homologues in the mouse model. Histone deacetylase inhibitor drugs disrupt this complex, restore target gene expression and lead to cell death. Tissue microarrays reveal consistent and high ATF2 nuclear expression in synovial sarcoma, with only solitary fibrous tumor showing higher levels. However, its expression is not as specific as TLE1 as relatively high levels are also seen in melanoma/clear cell sarcoma, Ewing tumors and MPNST. Levels in other mesencymal tumors and carinomas are significantly lower.

Conclusions: The ATF2-SS18SSX-TLE1 complex is present in patient synovial sarcoma specimens. ATF2 directs SS18-SSX to target genes in synovial sarcoma, which become repressed instead of activated. While not as useful a diagnostic marker as TLE1, the combined presence of ATF2 and TLE1 in a complex explains a mechanism of action for SS18SSX, and suggests means of therapeutic intervention for which clinical trials have recently opened.

74 MYH9-USP6 Fusion Transcript in Nodular Fasciitis: An Institutional Review

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Background: Nodular fasciitis (NF) is a benign, self-limiting proliferation of fibroblasts and myofibroblasts. The rapid growth, rich cellularity, and brisk mitotic activity that can be present in these lesions may pose a diagnostic challenge in the context of limited sampling, as with needle core biopsies. In a recent report, a majority of cases of NF were shown to harbor a stable translocation between ubiquitin specific peptidase 6 (USP6) on chromosome 17 and myosin heavy chain 9 (MYH9) on chromosome 22. The purpose of this study was to perform an institutional review of cases diagnosed as NF to assess for the presence of the MYH9-USP6 fusion product.

Design: Ribonucleic acid was extracted from archival formalin-fixed, paraffin-embedded tissue from 22 cases of NF and 26 cases of non-NF soft tissue tumors (including: benign fibrous histiocytoma, Ewing family tumor, ischemic fasciitis, myositis ossificans, myxofibrosarcoma, myxoid liposarcoma, non-ossifying fibroma, proliferative myositis, solitary fibrous tumor, and synovial sarcoma). Reverse transcriptase-polymerase chain reaction (RT-PCR) was performed using primers specific for the breakpoint between exon 1 of MYH9 and exon 2 of USP6.

Results: The MYH9-USP6 fusion transcript was identified in 19 of 22 (86%) NF cases. No fusion product was detected in any of the 26 non-NF cases (0%). All PCR products were verified by direct sequencing analysis. In comparing the morphology of lesions with and without the translocation, there were no conspicuous differences.
Conclusions: This study confirms the presence and specificity of the MYH9-USP6 gene fusion in NF, and these results demonstrate this can be exploited in a diagnostic assay suitable for use on formalin-fixed, paraffin-embedded tissue. It is possible that cases of NF that were negative for the fusion product have breakpoints not identified by this assay; this requires confirmation by fluorescence in situ hybridization. Recurrent chromosomal translocations characterize numerous malignant, and far fewer benign soft tissue tumors; NF is the only lesion, however, that frequently is associated with spontaneous regression. Further research is necessary into the pathogenesis of this unique self-limiting neoplasm, and the contribution, if any, of the fusion product in limiting growth of these lesions.

75 Assessment of MUC4 Expression in Primary Bone Sarcomas

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Background: Mucin 4 (MUC4), a glycoprotein expressed in various types of carcinomas, has been recently highlighted as a specific and sensitive immunohistochemical marker of low grade fibromyxoid sarcoma (LFS), a soft tissue tumor the hallmark of which is the t(7;16)(q33;p11). Since it is recognised that osteosarcoma - the most frequent primary bone sarcoma - can mimic virtually all other tumors thereby making them difficult to diagnose, particularly on needle cores, we sought to analyse the expression of MUC4 on a series of primary malignant bone tumors, as such data are not available in the literature. Review of our pathology archive revealed that we had previously reported 2 tumors with morphological features consistent with LFS and the closely related epithelioid sclerosing fibrosarcoma (ESF), a tumor which has been rarely reported as occuring in bone.
Design: To address the sensitivity and specificity of MUC4 expression in sarcomas, in addition to primary bone tumors we included a series of soft tissues neoplasms, some of which may rarely arise in bone. Tissue microrrays, prepared at our Institution, including 120 osteosarcomas, 220 chondrosarcomas, 45 chordomas, 60 myxofibrosarcomas, 54 solitary fibrous tumors, 59 leiomyosarcomas and 90 synovial sarcomas were studied. Whole tissue sections of 8 ossifying fibromyxoid tumors and the 2 cases with features consistent with ESF (*FUS*-rearrangement negative) and LFS (*FUS*-rearrangement not informative) were analyzed. For comparison, 8 cases of ESF of soft tissue (*FUS*-rearrangement negative) were added. Ten *FUS*-rearrangement positive LFS were used as control. Immunohistochemistry was performed using standard methods.
Results: All LFS with the characteristic t(7;16) expressed MUC4 diffusely. The 2 primary ESF/LFS of bone were diffusely positive for MUC4. Three out of 8 ESF of soft tissue (37.5%) were diffusely immunoreactive for MUC4 and 22/90 SS (24.4%) were focally positive. All the remaining tumors were negative for MUC4.
Conclusions: We have identified 2 cases of *bona fide* primary malignant bone tumors that displayed morphological features consistent with LFS (scapula) and ESF (femur), which diffusely express MUC4. 25% of SS expressed MUC4 focally, mostly byphasic type, thereby confirming previously published data. We conclude that once a diagnosis of SS is excluded, it is very unlikely that tumors in bone other than LFS and ESF, express MUC4. We recommend that MUC4 expression is studied in all fibromyxoid and sclerosing tumors of bone, in order to improve the classification of primary bone tumors, as this may help stratify patients for neo-adjuvant treatment.

76 Utility of a Monoclonal ERG/FLI1 Antibody for Immunohistochemical Discrimination of Ewing's Family Tumors

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Background: Ewing family tumors (EFTs; Ewing sarcoma/peripheral neuroectodermal tumors) and prostate carcinomas (PCa) are characterized by recurrent rearrangement of ETS family members, including *FLI1* (EFTs), *ERG* (PCa>>EFTs) and less commonly *ETV1*, *ETV4* and *ETV5*. Previously, we characterized an antibody against ERG (EPR3864) as showing diagnostic utility for the detection of ERG rearranged PCa. More recently, this antibody was shown to cross react with FLI1; thus, here we evaluate the utility of EPR3864 immunostain for discrimination of EWTs from other small round blue cell tumors (SRBCTs).
Design: Western blotting using EPR3864 was performed on EFT cell lines harboring EWS:ERG or EWS:FLI1 fusions. ERG/FLI1 staining by immunohistochemistry (IHC) was evaluated on a TMA with 111 EFT cases from 89 patients, on individual sections of 2 additional EFTs, and on 61 other SRBCTs. Nuclear ERG staining was scored as 0 (negative), 1+ (weak), 2+ (moderate) and 3+ (strong). Staining of vessels was used as a positive control. EFTs were also evaluated for CD99 expression, which was scored as negative or positive (cytoplasmic or membranous).
Results: By Western blotting using EFT cell lines, we demonstrate that EPR3864 detects both EWS:FLI1 and EWS:ERG. Of 53 EFTs evaluable by IHC, 44 (83%) demonstrated at least 2+ diffuse nuclear ERG/FLI1 staining, of which 1, 4 and 39 showed negative, cytoplasmic or membranous CD99 staining, respectively. Of the remaining 9 EFTs with 0-1+ ERG/FLI1 staining, 3, 2 and 4 showed negative, cytoplasmic or membranous CD99 staining, respectively. Amongst other SRBCTs, at least 2+ diffuse nuclear staining was seen in 0 of 11 (0%) Wilm's tumors, 0 of 11 (0%) neuroblastomas, 0 of 7 (0%) alveolar/embryonal rhabdomyosarcomas, 0 of 4 (0%) desmoplastic small round cell tumors, 1 of 10 (10%) Burkitt's lymphomas, 5 of 11 monophasic synovial sarcomas (45%) and 7 of 7 (100%) of precursor-B-lymphoblastic lymphomas/leukemias.
Conclusions: EPR3864 has high sensitivity for EFTs, consistent with reported rates for other anti FLI1 polyclonal and monoclonal antibodies, and is highly correlated with CD99 expression. Amongst other SRBCTs, EPR3864 also stained all precursor-B-lymphoblastic lymphomas/leukemias and a subset of Burkitt's lymphomas and synovial sarcomas, suggesting the need for additional IHC/molecular tests to differentiate these diagnoses. Our results suggest that EPR3864 may have utility in the differentiation of EFTs from other SRBCTs, in addition to its reported utility in PCa.

77 Melanotic Schwannoma (MS): A ClinicoPathologic Study of 32 Cases

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Background: MS, variably associated with Carney Syndrome (CS), are rare tumors that usually involve spinal nerve roots but may occur in other locations. Clinicopathological evaluation poorly predicts the behavior of MS. Fewer than than 200 cases have been reported. We report a series of 32 well-characterized MS, the largest series to date.
Design: 32 MS were retrieved and evaluated for growth pattern, nuclear atypia, cellularity, mitotic rate, and necrosis. Follow-up was obtained. Immunohistochemistry (IHC) for S-100 protein, melan A, HMB 45, tyrosinase, INI-1, glial fibrillary acidic protein (GFAP) and Ki-67 antigen was performed using commercially available antibodies and the Dako Envision detection system. Fisher's Exact Test was used for statistical analysis.
Results: The tumors occurred in 15 males and 17 females (mean age 42 years, range 11-84 years). Tumors occurred in paravertebral nerve roots (N=27), mediastinum (N=2), 5th cranial nerve, buttock, and cerebellum (N=1 each). No patient had a definite history of CS although 1 patient also had a cutanteous myxoma and is presumed to have CS. IHC was S100 protein (16/20, 80%), Melan A (18/20, 90%), HMB45 (20/20, 100%), tyrosinase (20/20, 100%), GFAP (0/20, 0%), and INI1 (retained in 20/20, 100%). Ki-67 index was <5% (18/20, 90%) and 5-10% (2/20, 10%). Clinical follow-up (19/32, 59%, mean 5.4 years, range 1 month-25 years) showed local recurrences in 7/19 (36%) and metastases in 9/19 (47%) patients. Metastastic sites included lung, pleura, liver, lymph nodes, leptomeninges, brain and soft tissues. The only feature that correlated with metastases was mitotic rate >2/10 high powered fields (p=0.03). Patients were alive without disease (N=8), alive with disease (N=7) and dead of disease (N=4).
Conclusions: Our study confirms the unpredictable and potentially aggressive nature of MS, with local recurrences and distant metastases in 36% and 47% of patients, respectively. The presence of elevated mitotic activity may help to predict those patients at greater risk for the development of metastases. A subset of MS lack S100 protein expression, an unusual finding in a neoplasm of putative schwannian origin. Although previous studies have clearly shown an increased incidence of MS in patients with CS, many MS occur in patients not known to have this syndrome. On-going evaluation of MS for loss of the CS-associated *PRKAR1A* tumor suppressor gene and DNA microarray study should help to clarify the relationship of MS to CS and to other melanocytic/nerve sheath tumors, respectively.

78 Recurrent Amplification at 7q21.2 Targets *CDK6* Gene in Primary Myxofibrosarcomas and Identifies CDK6 Overexpression as an Independent Adverse Prognosticator

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Background: Myxofibrosarcoma is genetically complex and remains obscure in molecular determinants of clinical aggressiveness. Our prior array comparative genomic hybridization study on myxofibrosarcomas revealed recurrent gains of 7q where *CDK6* gene displayed increased DNA copies.
Design: On tissue microarrays, CDK6 immunoexpression was assessable in 77 primary tumors, 55 of which were successfully determined for *CDK6* gene dosage by real-time PCR using genomic DNA extracted from laser-microdissected tumor cells. Gene status and protein expression of CDK6 were correlated with each other, clincopathological variables, metastasis-free survival (MFS), and disease-specific survival (DSS).
Results: Detected in 21(27.2%) of 77 and 13(23.6%) of 55 cases, respectively, protein overexpression and gene amplification of CDK6 were highly related to each other (p<0.001) and associated with higher grades (overexpression, p=0.004; amplification, p=0.014). Univariately, both protein overexpression (MFS, p=0.0002; DSS, p=0.0015) and gene amplification (MFS, p=0.0001; DSS, p=0.0083) were significantly associated with worse outcomes. Importantly, CDK6 overexpression independently portended poorer MFS (p=0.0015, risk ratio [RR] =7.411) and also inferior DSS (p=0.0069, RR =6.006).
Conclusions: In approximately a quarter of primary myxofibrosarcomas, CDK6 overexpression is mostly driven by gene amplification on 7q, associated with adverse prognosticators, and independently predictive of worse outcomes, highlighting its possible causative role in tumor aggressiveness.

79 Skeletal Metastases: An Analysis of Patients with Unknown Primary Site

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Background: Pathologic evaluation of bony metastases in cases with prior history malignancy is usually straightforward. However, when a patient does not have a known malignancy, nor a complete radiologic work-up, pathologic evaluation of bony metastases is more challenging, becoming a process of elimination of potential primary sites. In our study we aim to investigate if there are certain common features characteristic for this subset of bony metastases. Having knowledge of the malignancies more likely to produce bony metastases before manifesting at the primary site helps the pathologist to be more efficient in the work-up of these cases.

Design: All cases of bony metastases diagnosed at our center between 2005-2010 were retrieved. Hematologic malignancies were excluded. Site of involvement, clinical and radiologic data, history of malignancy, and pathologic diagnosis were recorded. Sites of involvement were: central (axial skeleton), proximal (above elbows/knees), and distal (below elbows/knees). Information collected on patients with bony metastases and unknown primary site was compared to that of patients with bony metastases and known malignancy.

Results: 119 bone metastases were identified and 40 cases (33.6%) had no known primary tumor at the time of presentation. Our data suggests that skeletal metastases in this group are more likely to be adenocarcinomas and central in location.

Table 1. Skeletal metastases with unknown primary site versus known primary site

		Cases with unknown primary site (40)	Cases with known primary site (79)
Site	Central	21 (52.5%)	31 (39.2%)
	Proximal	13 (32.5%)	42 (53.2%)
	Distal	6 (15%)	6 (7.6%)
Pathologic fracture	Present	15 (37.5%)	33 (41.8%)
	Absent	25 (62.5 %)	46 (58.2%)
Pathologic diagnosis	Adenocarcinoma	22 (55%)	34 (43.1%)
	Squamous cell carcinoma	6 (15%)	9 (11.4%)
	Poorly differentiated carcinoma	3 (7.5%)	5 (6.3%)
	Renal cell carcinoma	5 (12.5%)	13 (16.4%)
	Thyroid carcinoma	1 (2.5%)	6 (7.6%)
	Other	3 (7.5%)	12 (15.2%)

The most likely primary sites were lung (30%) and kidney (12.5%). However, in 37.5% cases a primary site could not be determined based on pathology alone. Bony metastases in cases with prior history of malignancy were from lung (26.6%), breast (25.4%), kidney (16.4%), prostate (8.8%), thyroid (7.6%), or other (15.2%).

Conclusions: In patients with skeletal metastases and unknown malignancy at presentation the two most likely primary sites are lung and kidney, however in a large proportion of cases the primary site cannot be determined by pathologic evaluation alone.

80 Transforming Growth Factor-ß and Connective Tissue Growth Factor Are Mitogenic Output Mediators of Wnt/ß-Catenin Signalling in Deep Fibromatosis

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Background: Deep fibromatosis (includes mesenteric, abdominal and extraabdominal fibromatosis) are clonal proliferations of fibroblastic and myofibroblastic cells that are locally aggressive with high recurrence rates and no metastatic potential. Implicated molecular aberrations occur within the Wnt/beta-catenin pathway (APC and ß-catenin gene mutations). Transforming Growth Factor-beta (TFG- ß) and Connective Tissue Growth Factors (CTGF) are profibrotic growth factors, down stream from nuclear translocation of ß-catenin, that lead to increased fibrogenesis. CTGF (a downstream effector of TFG-ß) is a matricellular protein that modulates the activity of growth factors, adhesion molecules, integrins and extracellular matrix (ECM) thus playing a central role in tissue remodelling and fibrosis. It is plausible that given this role that CTGF might represent an important mediator in the pathogenesis of deep fibromatosis (DF). Recently there has been growing interest in use of ECM inhibitors for treatment of various fibrogenic diseases. Monoclonal antibodies targeting this are currently in clinical trials. Herein, a cohort of DF is evaluated for expression of ß-catenin, TGF-ß and CTGF using immunohistochemistry.

Design: 15 cases of DF were immunostained with TGF-ß, CTGF and ß-catenin biomarkers. Control group with 10 cases of normal skin and adjacent scar tissue were simultaneously immunostained with above mentioned markers. Using semiquantitative evaluation, any intensity of staining in >5% of the area of interest was considered positive (nuclear staining for ß-catenin, cytoplasmic staining for TGF-ß and CTGF).

Results: 15 of 15 DTs were positive for ß-catenin stain which was negative in control normal skin and scar. TGF-ß and CTGF were negative in 9 of 10 normal skin controls. TGF-ß were positive in 9 of 10 scar tissues and CTGF was positive in all cases of scar tissue. All 15 cases of DTs were positive for TGF-ß and CTGF There did not appear to be a difference in CTGF expression between DF and scar.

Table 1: Positive staining with biomarkers (in%)

	TGF-ß	CTGF	ß-catenin
Normal skin (n=10)	10	10	0
Scar tissue (n=10)	90	100	0
DF (n=15)	100	100	100

Conclusions: All cases of DF showed ß-catenin expression (suggesting Wnt/ ß-catenin pathway dysregulation) and high constitutive expression of downstream effectors; TGF-ß & CTGF. Myofibroblastic cells of scar tissue showed high expression of TGF-ß & CTGF but no expression of ß-catenin indicating different mechanisms. Constitutive expression of CTGF in DF has potential for enabling targeted therapy.

81 Expression of ERG, an Ets Family Transcription Factor, Specifically Identifies *ERG*-Rearranged Ewing Sarcoma

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Background: *ERG* encodes for an Ets family regulatory transcription factor and is involved in recurrent translocations in some acute myeloid leukemias, prostate carcinomas and Ewing sarcomas (ES). The purpose of this study was to examine the utility of an ERG antibody to detect *EWSR1-ERG* rearranged ES.

Design: Formalin-fixed paraffin-embedded tissue microarray and whole tissue sections of 33 cases of genetically defined ES were examined: 22 with *EWSR1-FLI1*, 9 with *EWSR1-ERG* and 2 with *EWSR1-NFATC2*. Immunohistochemistry was performed using a rabbit anti-ERG monoclonal antibody directed against the C-terminus (1:2000; EPR3864(2); Epitomics) and a mouse anti-FLI1 monoclonal antibody against a FLI1 Ets domain (C-terminus) fusion protein (1:100; G146-222; BD Biosciences). Immunoreactivity was graded for extent and intensity of positive tumor cell nuclei.

Results: ERG labeling was seen in 8/9 *EWSR1-ERG* cases (predominantly diffuse, moderate to strong), while only 4/24 non-*EWR1-ERG* cases showed labeling (very weak). FLI1 labeling was observed in 29/32 cases regardless of fusion variant; 23 displayed diffuse strong/moderate labeling (5/8 *EWSR1-ERG*, 18/21 *EWSR1-FLI1*). Both *EWSR1-NFATC2* cases had weak reactivity with both FLI1 and ERG.

Conclusions: Strong nuclear ERG immunoreactivity is specific for ES with *EWSR1-ERG* rearrangement. In contrast, FLI1 is not specific to rearrangement type, perhaps due to cross reactivity with the highly homologous Ets DNA binding domain present in both ERG and FLI1. Reagent selection is critical; antibodies against the C-terminus must be employed, as the N-terminus of the ERG or FLI1 protein is substituted by domains of the EWSR1 protein in the active fusion product.

82 MDM2 Copy Numbers in Well Differentiated and Dedifferentiated Liposarcoma: Where Do We Draw the Line?

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Background: MDM2 gene amplification is a known molecular abnormality associated with specific liposarcomas. Most recently, this amplificiation has been shown to be helpful in differentiating benign lipoamtous neoplasms from well differentiated (WDL) and dedifferentiated liposarcomas (DDL). A ratio of MDM2 copy number to CEP12 signal greater than 2 has been shown to be diagnostic of amplification and malignancy. What has not been established is the trend in variation of MDM2 copy numbers between WDL and DDL. The purpose of this study is to determine if DDL has higher amplification of MDM2 copy numbers as compared to WDL using FISH analysis.

Design: A total of 50 samples were analyzed; 26 WDL, 14 DDL and 10 control cases. The cases were reviewed for appropriate diagnosis. We also looked at borderline areas, myxoid areas and areas of possible low grade dedifferentiation. FISH for MDM2 and CEP12 was conducted on all specimens. The total MDM2 copy number per cell as well as the ratio of MDM2 copy number to CEP12 signals, were determined for each case. Negative controls included benign lipomatous neoplasms as well as mxyoid liposarcomas.

Results: 50 samples were reviewed from 43 patients, 27 men and 16 women and a mean age of 59 (22-86). MDM2/cell in the WDL cases had a mean of 24.5 (5.2-39.14) and the DDL cases had a mean of 39.5 (29.5-60) [p-value= 0.0002]. The MDM2/CEP12 ratio in the WDL cases had a mean of 12.1 (2.4-22.2) and the DDL cases had a mean of 20 (17-27) [p value= 0.00001]. Interestingly, in evaluating borderline areas when compared to WDL, albeit the small number of cases studied (n=4), they also showed significantly higher rate of MDM2 amplification (mean MDM2/cell = 35.7; mean ratio = 19.5). All negative controls showed no amplification of the MDM2 gene (MDM2/CEP12 ratio<2).

	Mean MDM2/Cell [CI]	Mean MDM2/CEP12 Ratio [CI]
WDL	24.5 [21-28]	12.1 [9.7-14.5]
DDL	39.5 [34.4-44.6]	20.0 [17.8-22.2]
Borderline	35.7	19.5

CI: confidence interval

Conclusions: Dedifferentiated liposarcomas have significantly increased amplification of MDM2 copy numbers as compared to well differentiated liposarcomas using FISH techniques. Both well-differentiated and de-differentiated liposarcomas can show variable morphology and have potentially overlapping characteristics. Initial observations of increased MDM2 amplification within borderline areas of liposarcoma are promising and provide a future area of focus. Further studies comparing not only morphology but also MDM2 copy numbers with clinical outcomes could help better define the boundaries between these two entities.

83 MicroRNA Profiles in Osteosarcoma

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Background: microRNAs (miRNA) are evolutionarily conserved small non-coding RNAs, which regulate gene expression by translational inhibition or cleavage of target mRNAs. The miRNAs play important roles in the development, differentiation and fuction of various cell types, and in the pathogenesis of various human diseases. The miRNAs are differentially expressed in normal cells and cancer cells.

Design: We performed a miRNA microarray analysis on 8 formalin fixed paraffin embedded osteosarcoma tissue samples and two osteosarcoma cell lines (SJSA-1 and MG63). And we confirmed the result of microarray by reverse transcription polymerase chain reaction.

Results: The data from miRNA profiling of osteosarcoma indicated that 10 miRNAs showed 10-fold increased expression compared to normal control. Among the 10 miRNAs, three miRNAs (miR-199b-5p, miR-338-3p, and miR-891a) were confirmed with reverse transcription polymerase chain reaction. The miR-199b-5p, miR-338-3p, and miR-891a were increased by 13.6-fold, 6.2-fold, and 6.8-fold respectively.

Conclusions: The profiles of miRNAs in the osteosarcoma is largely unknown. The investigation of the miRNAs expression between normal and osteosarcoma is crucial step for future clinical trials. The miRNAs have been recently shown to be useful tools to silence cancers. The miR-199b-5p is known to be involved in Notch pathway, targeting HES1. And miR-199b-5p expression is low in metastatic medulloblastoma patients. And miR-199b-5p expression specifically impairs the caner-stem-cell population, which results in impairment of medulloblastoma tumor development in the cerebellum xenograft mouse model. Recent report showed that miR-338-3p contribute to the formation of basolateral polarity in epithelial cells. These results revealed a

potentially important role for miRNAs in the process of epithelial cell differentiation. The disruption of polarity in epithelial cells is associated with poor prognosis for carcinomas. However, in mesenchymal tumor such as osteosarcoma, the miRNAs function is largely unknown. The miR-891a is known to be associated with BLCAP (bladder cancer associated protein). This gene encodes a tumor suppressor protein that reduces cell growth by stimulating apoptosis. Further studies about the specific function of three miRNAs in osteosarcomas is needed.

84 ALK Immunoexpression and Gene Status in Rhabdomyosarcomas

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Background: Anaplastic lymphoma kinase (ALK) is overexpressed via gene alteration in a number of neoplasms, and it has recently become a promising target of a specific inhibitor. Although ALK expression is immunohistochemically detectable, recent studies on *ALK*-rearranged lung cancer showed that staining according to the conventional protocol is unreliable. As a result, several sensitive staining methods have been developed. ALK expression in rhabdomyosarcoma (RMS) has been reported in a few studies, but sensitive staining method has not been tested. In addition, the genetic basis of ALK expression in RMS remains poorly understood.

Design: We performed a previously validated (Am J Surg Pathol. 2011:35;1224-38) sensitive ALK immunostaining for 106 RMSs (35 embryonal, 56 alveolar [ARMS], 7 pleomorphic, 8 adult-spindle/sclerosing). The staining results were correlated with the clinicopathological findings and *FOXO1* status studied by FISH. Selected cases were also tested for *ALK* rearrangement (with *ALK* break-apart probes) and copy number change (with *ALK/CEN2* probes) by using FISH and for *ALK* somatic mutation by PCR and sequencing.

Results: ALK expression was identified in 2 (5.7%) embryonal, 38 (68%) alveolar, and 0 (0%) pleomorphic and spindle/sclerosing types. Staining was diffuse (>50%) in most (90%) positive cases. ALK-positive ARMS more commonly presented with metastasis than ALK-negative ARMS ($p < 0.01$). *FOXO1* rearrangement was present in 38/43 of the ARMS, and all the 5 *FOXO1*-wild-type ARMSs were ALK-negative. *ALK* rearrangement was absent in all the tested ALK-positive cases (0/12). Gene amplification (median *ALK/CEN2* ≥ 2), low-level gain (2 > median *ALK/CEN2* > 1), and high polysomy (≥ 4 *ALK* copies in >40% of cells) were identified in 1, 3, and 9 of the 48 successfully studied cases, respectively, and these were all positive for ALK expression. Mutation was present in 1 of the 19 successfully studied cases, but the mutated tumor was an ALK-negative embryonal type.

Conclusions: ALK expression is relatively specific to the alveolar type and seems limited to the *FOXO1*-rearranged subset. Positive staining in ARMS may indicate a subgroup with a proclivity to early metastasis. ALK copy number change is related to protein expression, but this was observed in only a subset (~30%) of the immunopositive cases. ALK gene rearrangement and mutation do not seem to play a major role in RMS. These data may help to preselect patients with RMSs for ALK-targeted therapy in future clinical trials.

85 Comprehensive Analysis of Cathepsin K Expression in Human Neoplasms

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Background: Cathepsin K is a papain-like cysteine protease which is responsible for degradation of collagen type 1 and other bone proteins. Cathepsin K is expressed in osteoclasts under the control of Microphthalmia Transcription Factor (MiTF), and has been shown to be expressed in melanoma which is also MiTF positive. We have recently shown that Cathepsin K is consistently and diffusely expressed in alveolar soft part sarcoma (ASPS) and a subset of translocation renal cell carcinomas (RCC) which overexpress gene fusions involving the related transcription factors TFE3 and TFEB, but is not expressed in conventional RCC. However, a systemic analysis of Cathepsin K expression in human neoplasms, particularly those in a differential diagnosis of ASPS and translocation RCC, has not been performed.

Design: We constructed tissue microarrays (TMA) from a wide variety of human neoplasms, spanning approximately 9000 spots from 1562 samples derived from 72 different tumor types. The TMA were labeled for Cathepsin K by immunohistochemistry. Labeling was scored for percentage labeling (0-100%) and intensity (0=none, 1=weak, 2=moderate, 3=strong), and these were multiplied to give an H-score (0-300). Labeling yielding an H score of 20 or more was considered positive.

Results: Only 2 of 956 carcinomas from various sites (0.2 %) were positive for Cathepsin K; almost all were completely negative. However, Cathepsin K was expressed in many non-epithelial lesions, some of which fall in differential diagnosis of ASPS. Notably, Cathepsin K was expressed in granular cell tumor (57% of cases), juvenile xanthogranuloma (78% of cases), and (as previously reported) melanoma (66% of cases). In contrast, clear cell sarcoma (12 cases), adrenal cortical neoplasms (36 cases) and paragangliomas (19 cases) were consistently negative for Cathepsin K.

Conclusions: Among carcinomas, Cathepsin K labeling is highly specific for translocation RCC. While it is a highly sensitive marker for ASPS, Cathepsin K labeling among soft tissue tumors is not specific, in that it is expressed in a variety of mesenchymal lesions, including some of those in morphological differential diagnosis of ASPS. In particular, Cathepsin K expression in granular cell tumor and histiocytic lesions warrants diagnostic caution. However, the absence of diffuse Cathepsin K expression in clear cell sarcoma, adrenal cortical neoplasms and paraganglioma can help distinguish these 3 lesions from ASPS.

Breast

86 Breast Cancer HER2 Heterogeneity by FISH Pre and Post Neoadjuvant Chemotherapy: A Pilot Study

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Background: In 2009 a College of American Pathologist expert panel (CAP-EP) published recommendations for reporting HER2 "heterogeneous" cases in which 5-50% of individual cells are amplified for HER2 by fluorescence in situ hybridization (FISH). As a pilot study to examine the potential implications of HER2 heterogeneity by these criteria, we examined a series of cases before and after neoadjuvant chemotherapy to see if minor populations of amplified cells changed with therapy.

Design: HER2 FISH was performed on 34 cases of locally advanced breast cancer, including 17 biopsies and their matched surgical excision specimens after treatment with neoadjuvant chemotherapy. The percentage of cells with HER2 amplification by HER2:CEP17 individual cell ratio (ICR) and HER2 individual signals per cell (ICS) were analyzed and compared between the pre-neoadjuvant and post-neoadjuvant specimens.

Results: Based on the 2009 CAP-EP guidelines for HER2:CEP17 ICR, 47% (8/17) of patients had HER2 heterogeneity either pre or post chemotherapy (see below table). The percent amplified cells in these cases increased post-treatment in 63% (5/8) of these cases and decreased in 38% (3/8). Interestingly, 50% (4/8) of cases that had <5% amplified cells by ICR became heterogeneous in the post treatment specimen and one case became amplified (case 3 below). Using ICS criteria, heterogeneity was less common: 0% (0/17) of pre-neoadjuvant and 12% (2/17) of post-neoadjuvant cases had HER2 heterogeneity. Both ICS heterogeneous cases had increased HER2 heterogeneity in the post-neoadjuvant cases (2.5%→8% and 0%→37.5%).

Heterogeneity by HER2:CEP17 ratio (ICR)

	Pre-Neoadjuvant (N=17)	Post-Neoadjuvant (N=17)
Heterogeneous Cases:	% Individual Cells With Ratio >2.2	
1	25	4
2	11	0
3	8	63*
4	0	27.5
5	37.5	4
6	0	11.4
7	0	5
8	0	8
Cases With Heterogeneity:	24% (4)	24% (4)

*Amplified >50%; does not meet criteria for heterogeneity

Conclusions: The clinical significance of HER2 heterogeneity by FISH using the CAP-EP recommended criteria is still unclear. Minor populations of amplified cells can both increase and decrease post-chemotherapy and would be reported differently if ICR or ICS criteria are used. We plan to expand these studies to determine if there are more clinically and biologically relevant thresholds that should be used to report HER2 heterogeneity.

87 Metastatic Non-Small Cell Lung Carcinoma (NSCLC) Masquerading as Primary Breast Cancer (PBC) – A Rare yet Major Pitfall in Pathologic Diagnosis

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Background: PBC is the most common malignancy of women but metastatic malignancy to the breast has a reported frequency of 0.4 - 1.3%. The commonest non-mammary tumors (NMT) in the breast are hematological malignancies, malignant melanoma, lung tumors, renal cell carcinoma, ovarian tumors, and thyroid carcinoma. Accurate and timely diagnosis of metastatic NMT in the breast is mandatory to enable proper treatment. We compared clinical and pathological characteristics of metastatic NSCLC to breast with PBC to provide practical tools for pathologists in this essential differential diagnosis.

Design: Cases of non-hematopoietic NMT diagnosed in breast specimens were collected from archives of the BCCA Department of Pathology and the private collection of one author (MH). Clinical charts and pathologic slides were reviewed and ancillary tests performed where appropriate.

Results: 28 cases of metastatic NMT were identified including: 13 lung tumors, 6 melanomas, 4 ovarian tumors, 1 renal cell carcinoma, 1 vulvar carcinoma, 1 thymic carcinoma, 1 gastric carcinoma, and 1 carcinoid. NSCLC was the most common metastasis. Adenocarcinoma of lung (ACL) was most frequent (8/13), followed by small cell carcinoma (2/13). There was one case each of adenosquamous carcinoma, large cell neuroendocrine carcinoma and pulmonary carcinoid. The clinical and pathological features are summarized in table 1.

Table1: Clinicopathological characteristics of metastatic NSCLC to breast

Age/gender	Lung ca known at time of breast bx	Breast mass multifocality	DCIS	ER	TTF1	Axillary LNs	Distant metastases
64/F	Y	solitary	N	-	-	+	Y
70/F	N	two	N	+	+	+	NA
72/F	N	solitary	N	-	+	+	Y
76/M	N	two	N	-	-	-	Y
63/F	N	two, bilateral	N	-	+	+	Y
45/F	Y	solitary	N	-	+	-	Y
70/F	Y	solitary	N	-	NA	-	Y
69/F	N	solitary	N	-	+	+	Y
77/F	Y	solitary	N	-	-	NA	Y
65/F	N	solitary	N	NA	+	-	Y
55/F	Y	multiple bilateral	N	-	+	-	Y

Y=yes; N=no; NA=not available