Dermatopathology

483 Estrogen Receptor and Aromatase in Squamous Cell Carcinoma of the Skin

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Background: Estrogen has been known to be involved in development and/or progression of tumor cells arising in several organs including breast, endometrium, ovary, and others. Human skin is also considered the target organs of estrogens, which play some roles in the age-related dermal changes. In primary squamous cell carcinoma (SCC) of the skin, past history of burn and irradiation have been in general considered one of the important local carcinogenetic factors. In normal skin, fibroblasts in dermis have been demonstrated to express aromatase, one of the estrogen-producing enzymes, has also been proposed based on these findings but their details remain unknown. These injuries to skin above may also be considered to result in the disruption of estrogen-related physiological functions of the skin, which cause tumor. In this study, we therefore examined whether estrogen receptor (ER) α, ERβ and aromatase are expressed in SCC of the skin or not.

Design: Forty-eight cases (male: female 33:15, age range 44-99 yo.) with primary SCC of the skin were retrieved for imuunohistochemical study of ERα, ERβ, aromatase and Ki67. The specimens had been all fixed in 10% formaldehyde solution and embedded in paraffin. Serial tissue sections were used for hematoxylin-eosin staining and immunohistochemistry, performed by LSAB method. Nuclear immunoreactivity for ERα and ERβ was evaluated in 500 cancer cells, and the percentage of positive cells was subsequently obtained. Cytoplasmic immunoreactivity for armoatase with more than 10% of carcinoma cells was defined as "positive ". Ki67 labeling index was also obtained by counting 500 carcinoma cells. The statistical calculations, STATA version 7 (College Station, Texas) was used. P value under 0.05 was considered significant. Research protocols for this study were approved by Ethics Committee at University School of Medicine.

Results: ERα, ERβ and aromatase were positive in 93.75% (45/48 cases), 97.9% (47/48 cases) and 58.3% (28/48 cases), respectively. The positive rate of ERα among aromatase-positive cases was significantly higher among aromatse-negative cases ($p<0.05$, Mann-Whitney test). The age of aromatase-positive cases was significantly younger than aromatase-negative ones ($p<0.05$, Mann-Whitney test).

Conclusions: These results suggest that the combined expression of ERα and aromatase could be related to carcinogenesis and/or progression of SCC of human skin.

484 The MicroRNA Profile of Granular Cell Tumours

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Background: Granular cell tumors were first described by Abrikossoff in 1926 as lesions derived from smooth muscle, and referred to as granular cell myoblastoma. However, the exact histogenesis of the tumor is debatable, with some evidence suggesting a neural cell of origin. The purpose of this study was to examine the microRNA (miRNA) expression profile of granular cell tumors when compared to normal skin and to determine if the histogenesis of granular cell tumors can be clarified with their miRNA profile. MicroRNAs are small non-coding RNAs that down regulate gene expression in cellular apoptosis, differentiation, development, and appear to be expressed in a cell lineage specific manner.

Design: To address our question, we began by comparing the miRNA expression profiles of granular cell tumors (n=8) to normal skin (n=2) using archival formalin fixed, paraffin embedded (FFPE) tissue and the Agilent miRNA microarray platform. Then, using Genespring software, we found both up-regulated and down-regulated miRNAs in the granular cell tumors relative to normal skin.

Results: Firstly, our results showed that unsupervised hierarchical clustering was able to separate the granular cell tumors from normal skin. The three most significantly down-regulated miRNAs were: miR-203, showing a 315-fold change ($p \leq 0.05$), miR-200c, showing a 227-fold change ($p \leq 0.05$), and miR-200b, showing a 116-fold change ($p \leq 0.05$) in granular cell tumors when compared to their expression in normal skin. The three most significantly up-regulated miRNAs were: miR-370, showing a seven-fold change ($p \leq 0.05$), miR-490-5p, showing a six-fold change ($p \leq 0.05$), and miR-23a, showing a 5.6-fold change ($p \leq 0.05$) in granular cell tumors when compared to their expression in normal skin.

Conclusions: The reduction of the miR-200 family has been previously shown to correlate with epithelial-mesenchymal transition (EMT) and thus with granular cell tumors, this finding is in line with its mesenchymal differentiation. We plan to compare the granular cell tumor profile with that of smooth muscle and neural tissue to better elucidate its lineage. In summary, this is the first description of the miRNA profile of granular cell tumors, and this work may lead to a better understanding of the histogenesis of this enigmatic tumor.

485 Correlation of KIT Expression and KIT Gene Mutations in Acral Lentiginous and Mucosal Melanomas

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Background: KIT gene mutations have been reported in 19% of mucosal melanoma (MM) and 15% of acral lentiginous melanoma (ALM) cases. Since patients with gastrointestinal stromal tumours have a significant survival benefit following treatment with the tyrosine kinase inhibitor, Gleevac (imatinib mesylate, STI571), several clinical trials have offered Gleevac to patients with melanoma. Early results show a near complete response to treatment with Gleevac in patients harboring KIT gene mutations in either exons 11 or 13, irrespective of whether KIT expression was detected by immunohistochemistry (IHC). The goal of this project is to correlate KIT expression to KIT gene mutations in ALM and MM cases treated at the Sunnybrook Health Sciences Centre between 1990 to present date.

Design: Paraffin-embedded tissue from ALM and MM cases was retrieved from the archives. Histological assessment included routine H&E stains, as well as IHC staining for KIT. Genomic DNA extracted from tumor-rich areas was used for PCR-based amplification of exons 11 and 13. The PCR-amplified products were sequenced and analyzed for mutations.

Results: We have identified 60 ALM and MM cases, of which 78.33% are positive for KIT expression.

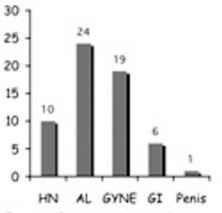

Figure 1. Site distribution of melanoma cases.

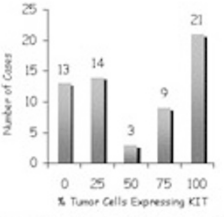

Figure 2. Melanoma cases positive for KIT expression.

Amplification of exon 11 has been successfully completed in 6 cases, 2 of which show the following mutations: M551K and N566D.

Conclusions: While studies have shown that KIT expression alone does not predict responsiveness to treatment with Gleevac, more recent clinical trials have demonstrated a near complete response to treatment in melanoma cases that harbor KIT gene mutations in exons 11 or 13. We hope that findings from our study will build upon the rationale for routine screening of patients with melanoma for both KIT expression and KIT gene mutations, possibly allowing for targeted treatment with either Gleevac or other tyrosine kinase inhibitors developed in the future.

486 Expression of Cancer Stem Cell Marker CD133 in Malignant Melanoma and Spitz Nevus in a Pediatric Population

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Background: Identification of cancer stem cell markers, such as CD133, may help refine classification, diagnosis and treatment of cancers including malignant melanoma. The role of CD133 protein as a factor serving in directing tumor growth is the subject of debate.

Design: We evaluated the immunohistochemical expression of CD133 in 12 malignant melanomas and in 20 Spitz nevi, diagnosed between 1990 and 2008, in our pediatric population. We reviewed the charts of these patients and noted their clinical evolution.

Results: CD133 was positive in 4 cases of our childhood malignant melanoma series. The latter were the only malignant melanoma cases that were associated with either lymph node or visceral metastases and/or death. CD133 was negative in all 20 cases of Spitz nevi.

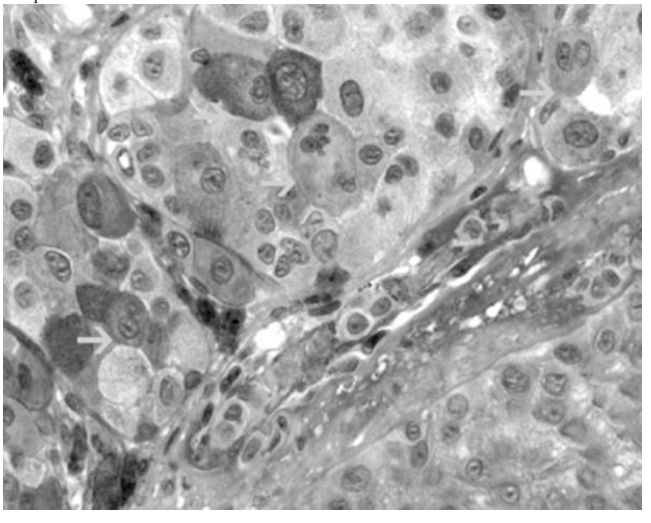

Figure 1: Positive CD133 (red) in multifocal melanoma cells, revealing clear cytoplasmic staining with focal membranous re-enforcement (green arrows), x 400 magnification.
Conclusions: CD133 positive cancer stem cell expression might correlate with bad prognosis (metastasis and/or death) in childhood malignant melanoma. Treatment targeting these cancer stem cells could decrease the chemoresistance of malignant melanoma in the pediatric population.

487 Virtual Microscopy in Melanoma Synoptic Reporting; a Validation and a Comparative Study

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Background: The current melanoma reporting relies heavily on proper synoptic reporting for more accurate, complete and reproducible reports. Aims: 1. To evaluate the current virtual microscopy (VM) in filling synoptic reports for melanoma cases. 2. To assess any possible impact of 20x scans vs. 40x scans on the accuracy.
Design: 10 consecutive cases of melanoma were collected. A total of 12 slides (10 H&E and 2 IHC slides) were scanned at both 20x and 40x using aperio scanscope CS. The cases were evaluated separately by 2 dermatopathologists. The results were evaluated.
Results: The melanoma cases were 1 in-situ, 2 nodular, 3 superficial spreading, one lentigo maligna melanoma, and 3 undetermined. Clark's level ranged from 1 to level 4. The melanoma thickness ranged from 0.0-4.3 mm (median 1.62mm). 6 cases were incompletely excised. There was one ulcerated case. None showed perineural or lymphovascular invasion or satellitosis. The mitotic index was reported in 7 cases ranging from 7-23/ mm ² with a median of 15.6. Pathologist A recorded the type of melanoma as originally reported in 9/10 cases. There was a discrepancy in 1 case were the type was recorded as superficial spreading instead of lentigo maligna melanoma. There was 100% concordance rate in Clark's level. The median thickness was 1.63 mm with no significant differences between 20x vs. 40x. All cases were reported as non-ulcerated missing one ulcerated melanoma. The margin status concordance rate was 100%. The mitotic index median was 5.4 (20x) vs. 6.0 (40x). Pathologist B reported melanoma types as originally reported in 9/10 cases with one case called lentigo maligna melanoma instead of superficial spreading melanoma. Clark's level estimation concurred with the original reports in all cases. The median thickness was 1.7 mm. One ulcerated melanoma was missed. The margin status concordance rate was 100%. The mitotic index median was 14 (x20) vs. 6.2 (x40).
Conclusions: The current system of virtual microscopy produces excellent quality images that make melanoma synoptic reporting feasible at both 20x and 40x scans. Although, in most cases 20x scans are sufficient, 40x scans can be resorted to in difficult cases for a far superior image quality. The current user interface (mouse) is not convenient specially when dealing with high volume. Also automated image analysis could play a major role in solving mitotic index evaluation, which inherently carries a high intra- and inter-observer variability.

488 Frequent Detection of Merkel Cell Polyoma Virus in Cutaneous Basal Cell Carcinoma

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Background: Polyomaviruses are frequently associated with malignancies in their hosts. Recently, a novel polyoma virus, named Merkel cell polyomavirus (MCV) was identified integrated to the genome of Merkel cell carcinoma (MCC) suggesting a role in its pathogenesis. Basal cell carcinoma (BCC) is the most common cutaneous neoplasm, characterized by basaloid cells with hyperchromatic nuclei and high nuclear to cytoplasmic ratio. We have observed that some BCC tumors show nuclear molding, finely granular chromatin pattern, high mitotic activity and expression of chromogranin, features that partially overlap with MCC however, in contrast to MCC are negative for CK20. In this study we evaluated the presence of MCV in a selected group of BCCs exhibiting morphologic or immunohistochemical neuroendocrine (NE) features.
Design: From our clinical database we selected 12 BCC cases that were deemed to exhibit NE morphology, and/ or expressed chromogranin. Following DNA extraction, we performed PCR using 2 prime pairs amplifying sequences within the T antigen of MCV. The identity of PCR products was confirmed by agarose gel electrophoresis. In addition, the selected cases as well as a control group of 44 BCC cases were evaluated for mitotic index / mm2, presence of necrosis, ulceration, apoptosis, squamous differentiation and peripheral palisading.
Results: All analyzed cases were negative for synaptophysin and CK20 ruling out MCC. Overall we were able to detect MCV amplicons in 9/12 analyzed tumors (75%). MCV positive cases compared to the rest of the cohort demonstrated a higher mitotic index (15 vs. 7 mitoses/ mm2, p=0.01), increase incidence of necrosis (37.5% vs. 12.5%, p=0.07), higher frequency of prominent apoptosis (50% vs. 12.5%, p=0.01) and less peripheral palisading (37.5% vs. 73%, p=0.04).
Conclusions: We have successfully identified the presence of MCV sequences in 75% of a subset of BCC tumors characterized by NE morphology and chromogranin expression. Our data shows that MCV is not specific for MCC tumors as initially described. MCV positive BCCs are characterized by higher mitotic rates, apoptosis, necrosis and lack of peripheral palisading. It is possible that MCV plays a role in the pathogenesis of both MCC and BCC tumors. Further studies are warranted to determine if the subset of MCV positive BCCs have a different biologic behavior.

489 The Embryonic Stem Cell Transcription Factor SOX2 Is Expressed by Spitz Nevi

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Background: Spitz nevi are benign melanocytic neoplasms that occur predominantly in children and young adults. Lesions with many histopathological features of Spitz nevi may occur in adults, and may display atypical architectural and cytological features. These lesions have been designated "atypical Spitz tumors" recognizing their potential for aggressive behavior. We previously found that the embryonic stem cell transcription factor SOX2 is expressed in a minority of banal nevi (15%) in contrast to malignant melanoma (42%). We sought to determine the expression of Sox2 in Spitz nevi and atypical Spitz tumors.
Design: Fourteen Spitz nevi, 6 atypical Spitz tumors and twenty primary cutaneous melanomas with 10-year follow-up were retrieved from the Pathology Service of the Massachusetts General Hospital. Twenty-one cases of banal nevi were retrieved from the archives of the Department of Pathology at Brigham and Women's Hospital. Five micron paraffin sections were stained with goat anti-human SOX2 immunoperoxidase antibodies (Neuromics, Edina, MN). Immunoreactivity was graded using a semiquantitative scale according to percentage of positive tumor cells as follows: (0 = negative, 1+ ≤ 5%, 2+ =6-25%, 3+ 26-50%, 4+ > 50%).
Results: Three out of 21 (14%) common nevi, 7/ 20 (30%) Spitz lesions (5/14 Spitz nevi and 2/6 atypical Spitz tumors), and 10/20 (50%) malignant melanomas were immunoreactive for SOX2, showing 1+ to 3+ staining. In terms of pattern, the common nevi had uniform diffuse positivity. Spitz showed positivity in random single cells or at the tumor periphery. The melanomas had either positive cells concentrated at the deep/advancing edge (8), in one group of larger cells (1), or randomly (1). No tumors expressed SOX2 in more than 50% of cells (4+). No significant association between SOX2 and presence of atypia in Spitz was found.
Conclusions: We confirm that SOX2 is expressed in a minority (14%) of benign common nevi in comparison to melanomas (50%), and identify SOX2 expression in 30% of Spitz nevi and Spitz tumors.

490 MITF Expression in Cutaneous Melanoma and Spitz Nevi: A Genomic and Immunohistochemical Study

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Background: Spitz nevi are spindled and epithelioid cell melanocytic proliferations that may histologically mimic melanoma.
Design: In an effort to further define the genomic profile of these tumors, we used a branched chain DNA assay to measure RNA in formalin fixed tumors from 40 patients. We evaluated twenty-five genes determined to be of interest based on prior work and current collaborations. We then examined the protein expression of the most robust discriminator of Spitz nevus versus melanoma.
Results: Twenty cases of Spitz nevus and 20 cases of vertical growth phase melanoma were evaluated. Of the 20 patients with Spitz nevi, ages ranged from 4 to 65 years (mean 26). All 20 patients were alive without evidence of disease, with a mean follow up of 11 years. Of the 20 patients with melanoma, the patient ages ranged from 20 to 77 years (mean 57). Twelve patients diagnosed with melanoma were without recurrence or metastasis (mean follow up 11 years). Eight patients with metastases died of melanoma, with an average survival time of 3 years. MITF was found to express the most significant difference in RNA levels between Spitz nevus and melanoma ($p<0.01$), with higher expression in melanomas than Spitz. However, no significant association of MITF with survival was observed while IL24 showed the most robust association with survival ($p<0.04$). MITF protein expression was also evaluated immunohistochemically and revealed no discernible correlation with RNA levels. MITF was detected immunohistochemically in all 20 Spitz tumors and 17/20 (85%) melanomas. Diffuse MITF expression was observed in 16/20 (80%) Spitz nevi and 8/20 (40%) melanomas. Regional staining for MITF was observed in 2/20 (10%) Spitz nevi and 8/20 (40%) melanomas. Scattered MITF expression was the least frequently observed pattern of MITF, with 2/20 (10%) of Spitz nevi and 1/20 (5%) of melanomas. Among the novel metastasis genes, we also identified 6 exhibiting significant differential expression between Spitz and melanoma. This data will be presented.
Conclusions: In conclusion, in routinely processed tumor specimens, we identified 7 genes with differential RNA expression between Spitz nevi and melanomas including MITF; immunohistochemical detection of MITF was not revealed as a diagnostic or prognostic factor.

491 Role of Foxp3+ T Lymphocytes in Lymph Nodes with Mycosis Fungoides Involvement

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Background: The objective of our study was to elucidate the role of DNA mismatch repair defects and microsatellite instability markers and Foxp3+ CD4+ CD25+ regulatory T cells (Tregs) in lymph node involvement with mycosis fungoides and correlate them with clinical data and flow analysis.
Design: Archived paraffin blocks with lymph nodes from 30 patients with Mycosis Fungoides (1991-2001) were used to construct a tissue microarray (2 cores/case) using an adhesive tape embedding system. Nine benign lymph nodes were used as control. Immunohistochemical (IHC) staining using antibodies to FOXP3, MLH1, MSH2, MSH6, CD3 and CD4 was performed. Foxp3+ CD4+ cells in the interfollicular zone were semi-quantitatively estimated as <10%, 10-50% and >50%. Microsatellite

instability (MSI) markers were graded as absent/reduced or present with absence of any single marker classified as MSI present. Scanscope XT (Aperio, Vista, CA) was used to evaluate the microarray slides. SPSS software was used for analysis.
Results: 25/30 patients had skin involvement data available (Patch – 4/25; Plaque - 2/25; Tumor - 4/25 tumor; Erythroderma - 8/25; Sezary Syndrome - 7/25). 30 MF patients had either a diagnosis of dermatopathic lymphadenitis (DL ; 17/30) or cutaneous T cell lymphoma (CTCL; 13/30). Patients with CTCL in lymph nodes were more likely to have Sezary Syndrome (6/13), when compared to patients with DL ($p<.05$). National Cancer Institute-Veterans Administration (NCI-VA) staging on 29/30 cases showed 13 CTCL cases graded as either LN3or LN4; while DL cases graded LN1or LN2. Flow data was available in 31 cases (22 MF and 9 benign). 3/17 patients with DL and 8/13 with CTCL had abnormal flow cytometry results. A significant number of cases with CTCL had Foxp3 levels of less than 10%, when compared to DL ($p<0.05$). There was significant correlation with Foxp3+ levels on immunohistochemistry and CD25 percentage by flow analysis (21/30). All cases with FOX P3 results of <10% by IHC (3/19) had <2.0% CD25 positive cells. No difference was seen between benign controls and lymph nodes involved by MF in MSI markers.
Conclusions: Decrease in Foxp3+ Tregs correlates with lymph node involvement in patients with Mycosis Fungoides and possibly plays a role in disease progression. Larger studies with additional T cell lymphomas are underway to further elucidate the role of Foxp3+ Tregs and their contribution in disease progression.

492 Expression of DNA Double Stranded Break Repair Proteins in Melanoma

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Background: UV (ultraviolet) radiation has been linked to increased risk of skin cancer including melanoma. However, the mechanism of UV radiation in carcinogenesis is still unclear. A viable hypothesis is the accumulation of damaged DNA by way of UV radiation, leading to DNA double stranded breaks (DSB). When the DSB load has reached an irreparable sum, the cells activate the pathway of cell death or apoptosis. Previously, we determined that DNA DSBs, as detected by histone H2AX phosphorylation, are increased in human melanoma tissue. Hence, we aim to determine if the downstream effector of DNA DSB, namely the DNA repair proteins, are recruited/overexpressed in melanoma cells as well. Examination of the proteins; MDC1 (mediator of DNA damage checkpoint protein), RAD50, 53BP1 (p53 binding protein 1) and NBS1 (Nijmegen breakage syndrome protein 1), responsible for the initial steps of DSB DNA damage repair should provide insight to the questions proposed.
Design: For this preliminary study, 15 cases of each of the following lesions have been retrieved from our archives: invasive melanoma, melanoma in-situ, dysplastic melanocytic nevi and congenital nevi. Commercially available antibodies specific for MDC1, RAD50, 53BP1, and NBS1 will be employed. Routine immunohistochemical stainings are performed. The results will be interpreted by relying both on the quantitative and qualitative value of positive staining patterns in the nucleus.
Results: Although we are still in the process of analyzing the results, we have already noticed that some the the DNA damage repair proteins such as NBS1 are overexpressed in many of the melanoma while others are decreased.
Conclusions: This is the first known systematic study to determine whether the DNA damage repair pathway is activated in human melanoma cells. Previous studies from our group and others have recognized that histone H2AX phosphorylation, which is associated with DNA DSB, is enhanced in melanoma cells. Increases in DNA DSB suggest that the breaks are either not being repair properly or that the breaks are being repaired but the histone signaling is defective. In the context of these results, pursuit and determination of whether the initial steps in the DNA damage repair pathway are defective is underway. Our preliminary results suggest that at least one of the DNA damage repair proteins is not localizing to the nucleus; therefore, supporting the idea that the increase in DNA DSB may be due to a defective DNA DSB repair protein. A larger study is currently being performed to further validate this result.

493 T-Cell Receptor PCR from Separate Biopsies Distinguishes Granulomatous Dermatitis from Granulomatous Mycosis Fungoides

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Background: The presence of a granulomatous tissue reaction in association with cutaneous T-cell lymphoma (CTCL), in particular mycosis fungoides (MF), is rare but well-documented. The diagnosis of MF may be difficult in cases where an exuberant granulomatous inflammatory infiltrate obscures the neoplastic lymphoid infiltrate, mimicking a granulomatous dermatitis. In these cases, the clinical evolution of the disease process, coupled with the demonstration of a monoclonal lymphoid T-cell population, may assist in establishing a definitive diagnosis. The presence of a monoclonal T-cell population has been reported in several of the inflammatory dermatoses considered in the differential diagnosis of early MF, and serves as a potential diagnostic pitfall. The frequency of T-cell clonality (TCC) in granulomatous dermatitides has not yet been established.
Design: A search of our archives revealed 29 cases of granulomatous dermatitis with biopsies at two distinct sites. We obtained the clinical findings at presentation and long-term follow-up. These findings were correlated with T-cell receptor gamma chain rearrangement by polymerase chain reaction (TCR-PCR) to evaluate for clonality at two separate anatomic skin sites.
Results: Of the 29 cases of granulomatous dermatitis, 17 cases had clinical follow-up (mean range 20 months) and 12 were lost to follow-up. Diagnoses included granuloma annulare (12), sarcoidosis (3), leprosy (2), annular elastolytic granuloma (1), necrobiosis lipoidica (1), necrobiotic xanthogranuloma (1), granulomatous rosacea (1), granulomatous dermatitis, infection vs. drug (7) and histiocytic disorder, NOS (1). Twenty-five of the 29 cases of granulomatous dermatitis were negative for TCC by dual TCR-PCR. One case of necrobiotic xanthogranuloma showed an identical T-cell clone in two different biopsies sites. Three cases of granuloma annulare showed a T-cell clone in only one biopsy site.
Conclusions: These data suggest that dual TCR-PCR is a clinically useful technique in distinguishing granulomatous inflammatory dermatitides from granulomatous mycosis fungoides. The infrequent finding of a T-cell clone in a granulomatous dermatitis underscores the importance of clinicopathologic correlation and clinical follow-up with sequential biopsies in establishing a definitive diagnosis.

494 Intradermal Nodular Fasciitis – A Rare Lesion: Clinicopathologic Analysis of 21 Cases

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Background: Nodular fasciitis, a benign myofibroblastic proliferation that occurs in the subcutaneous tissues of the upper extremities, trunk, and head and neck of young adults, is not widely recognized to arise primarily within the dermis. The purpose of this study was to examine the clinicopathologic and immunohistochemical features of a series of intradermal nodular fasciitis.
Design: Clinical and pathologic features and immunohistochemistry were evaluated in 21 cases of intradermal nodular fasciitis retrieved from consult files, in some of which a diagnosis of sarcoma had originally been suggested. Clinical follow-up was obtained from medical records and referring physicians.
Results: 11 patients were female and 10 were male, with a median age of 27.5 years (range 8-77). 9 lesions arose on the trunk, 8 on the limbs, 4 on the head and neck. Pre-operative duration ranged from a few weeks to 12 months. Tumors presented as a solitary swelling, ulcerated or bleeding mass. Grossly, the lesions were solid, nodular, rubbery, or firm and 0.7 to 3 cm in greatest dimension. Histologically, the lesions were well-circumscribed, unencapsulated tumor nodules involving the reticular dermis in 13 cases and the deeper half of the dermis with superficial extension into the subcutaneous tissue in 6 cases. The epidermis was ulcerated in 11 cases. Tumors were composed of palely eosinophilic spindle cells with plump, spindle to oval nuclei, small nucleoli and indistinct cytoplasmic borders, arranged in short intersecting bundles, which in some areas exhibited a storiform pattern, set in a microcystic myxoid stroma. In a third of the cases, occasional multinucleate giant cells of osteoclastic type were identified. 3 cases were associated with extensive stromal hemorrhage, 3 cases showed keloidal hyalinisation, and in 2 cases there was metaplastic ossification. The median mitotic index was 2 per 10 hpf (range 1 to 6 per 10 hpf). Scattered collections of lymphocytes, less often plasma cells and extravasated red blood cells and hemosiderin were present. Immunostaining for smooth muscle actin was strongly positive in 8/9 cases while S-100 and caldesmon staining were negative in all cases examined. None of the lesions has recurred or metastasized.
Conclusions: Intradermal nodular fasciitis occurs most commonly on the trunk of young adults, appears to show morphologic features similar to nodular fasciitis at conventional sites and should not be confused with sarcoma.

495 Sporadic Cutaneous Angiosarcomas Generally Lack HIF1α; a Histologic and Immunohistochemical Study of 45 Cases

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Background: Cutaneous angiosarcoma (AS) is a rare malignant neoplasm of dermis composed of infiltrating endothelial cells. Hypoxia-inducible factor-1 (HIF1) is a transcription factor that mediates cellular homeostatic responses to hypoxia. We studied the utility of HIF-1-alpha as a marker or explanatory factor in cutaneous AS.
Design: Forty five cases of cutaneous AS were retrieved from the Soft Tissue and Dermatopathology Registries of the Armed Forces Institute of Pathology (AFIP). Cases were re-reviewed for inclusion based on patient folder, slides, and obtained IHC. IHC for HIF-1 alpha was performed on 18 cases with available material.
Results: Seventeen and 83% of cases were females and males, respectively. The mean age at presentation is 67 years (range, 27-88). Tumors presented most commonly in the scalp followed by lower leg, face, arm and thigh. Associated BCC was noted in one case. No history of other primary, lymphedema, radiation, or Thorotrast-induced AS present. The tumors ranged in size from 0.4 -9.5 (mean of 2.4 cm). Histologically, most tumors were vasoformative, with either solid architecture (n=35) or papillary endothelial hyperplasia like foci (n=7). All cases demonstrated infiltrative growth pattern, cytologic atypia and mitotic activity. Surface ulceration was present in 44% and solar elastosis in the majority of cases. Epithelioid morphology was present in 29% (n=13), and mild to moderate lymphocytic inflammatory response in 62% (n=28) cases. CD31 highlighted malignant endothelial cells in all cases and SMA was generally absent. HIF-1alpha was focally positive in the cytoplasm of 3/18 (17%) cases. Follow-up data was available on 4 cases: 2 died of disease within 4 years and 2 had recurrence within 2 years.
Conclusions: Cutaneous AS is largely found on the scalp of older patients. Requirement for diagnosis includes extravascular proliferation of atypical endothelial cells with mitotic activity in vasoformative, solid or papillary patterns. Absence of SMA, indicating lack of pericytes around vessels, indicates extravascular extension of tumor outside normal vessels. Cutaneous AS generally lacks HIF-1alpha. Accordingly, the hypoxic response pathway cannot be documented as a common mechanism of angiogenesis in this entity.

496 Psoriatic Alopecia/Alopecia Areata-Like Reactions Secondary to Anti-TNF Therapy. A Novel Cause of Non-Cicatricial Alopecia

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Background: With the increasing use of anti-tumor necrosis factor (anti-TNF) biologic drugs to treat autoimmune diseases the spectrum of side effects including cutaneous reactions are becoming more evident. A not well recognized side effect is the development of alopecia. There are a few case reports (mostly clinical) in the literature and little is known about the histopathological characteristics of such lesions. The aim of this study was to evaluate the histopathological features of alopecia in this setting.

Design: Three patients (all females; 21, 27 and 39 years old) who developed scalp alopecia during anti-TNF treatment for Crohn's disease were included in this study. Two of them also developed a skin rash clinically consistent with psoriasis outside the scalp. None of these patients had history of psoriasis. Four scalp punch biopsies (2 patients had 1 and one had 2 biopsies) and 3 biopsies from the body rash were available.

Results: Clinically, the 3 patients had large scaly patches of alopecia. All scalp biopsies revealed psoriasiform features on the surface and alopecia areata-like changes in the dermis. Surface changes varied from mild to well-established epidermal hyperplasia with confluent parakeratosis containing neutrophils to frank pustules. The dermis showed marked increased catogen/telogen and miniaturized hairs and peribulbar lymphocytic inflammation. Numerous plasma cells and eosinophils were seen in all cases. Biopsies from the body rash showed changes similar to plaque-like and pustular psoriasis but with eosinophils and plasma cells in the infiltrate. In 2 patients the lesions improved with topical treatment and anti-TNF therapy continued. In one patient the alopecia improved only after anti-TNF treatment was stopped.

Conclusions: Anti-TNF therapy related alopecia may closely mimic primary psoriatic alopecia or alopecia areata. Distinguishing histopathological features include the epidermal psoriasiform changes and numerous plasma cells that are not features of alopecia areata and plasma cells and eosinophils that would be unusual in primary psoriasis. An awareness of this new class of alopecia and clinical correlation are crucial for the distinction. A correct diagnosis can enable effective treatment of the alopecia while allowing in some cases anti-TNF therapy to continue.

497 Deletions in Merkel Cell Polyomavirus Are Frequent, and Spare the Retinoblastoma-Binding Region

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Background: Merkel cell carcinoma (MCC) is a rare cutaneous tumor that was recently shown to harbor a novel polyomavirus. Similar to other polyomaviruses, the Merkel cell polyomavirus (MCPyV) encodes a viral large T antigen. In other carcinogenic polyomaviruses, the large T antigen effects control of cell proliferation via inhibition of Rb. It is unclear, however, if the MCPyV large T-antigen plays a role in carcinogenesis of MCC. We provide evidence that the Retinoblastoma (Rb) binding domain of MCPyV large T antigen is important in MCC and that it acts to block the normal regulatory activity of Rb.

Design: The study cohort consisted of 41 cases of MCC, 32 of which have been previously shown by PCR to harbor MCPyV. Paraffin blocks were first punched and DNA extracted. MCPyV positive MCC cases were then mapped by PCR for viral deletions using a novel set of 23 overlapping primers with an average size of 270bp that span the viral genome. The resulting PCR products were detected by gel electrophoresis. In a subset of cases, the Rb binding region (LxCxE domain) of the viral large T protein was sequenced. The function of the large T protein was evaluated via immunohistochemistry (IHC), using a phospo-specific antibody that recognizes only the Ser807/811 phosphorylated form of Rb (pRb). As a comparison, we measured the cellular proliferation rate by Ki-67 IHC. Slides were scanned at 20X resolution and image analyzed to determine the percentage of positive cells (nuclear staining) in each case.

Results: We found that viral deletions were common in MCPyV, occurring in 25 of 29 cases with amplifiable viral genomes. The most common deletions involved the large T origin-binding domain, necessary for viral replication. The deletions uniformly spare the region encoding the LxCxE Rb-binding domain of the large T protein (not mutated in 16 of 16 cases tested by direct sequencing). The pRB/Ki-67 ratio was decreased in MCPyV positive compared to MCPyV negative cases (mean 1.2 vs 13.6, respectively).

Conclusions: MCPyV genomic deletions are frequent in MCC, but spare the Rb binding domain. They generally result in a truncated large T protein, but spare the LxCxE Rb-binding domain. These results are consistent with a model in which MCPyV exerts its oncogenic effects by large T LxCxE binding of Rb, leading to release of E2F and subsequent circumvention of the normal cyclinD mediated G1 checkpoint. Consequently, pRb levels remain low despite a high proliferative index.

498 Mass Spectrometry-Based Identification of Proteins in Archival Malignant Melanoma Tissue Samples

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Background: Genomic studies indicate that malignant melanoma (MM) has distinct molecular defects. However, it is difficult to predict the functional consequence of any particular gene mutation on tumor pathogenesis, progression or response to treatment. While genes contain the instructions for cellular assembly, it is through the actions of their encoded proteins that the functional characteristics of any tumor, including MM, are manifest. In addition, changes at the protein level do not always correlate with mRNA levels due to translational and post-translational modifications. Therefore, the identification of protein biomarkers that could refine the diagnostic and prognostic information gained from the clinical and histopathological features of MM, and aid in the development of novel targeted therapies is warranted. We aim to evaluate the feasibility of a mass spectrometry (MS)-based approach to discover proteins in formalin-fixed paraffin-embedded (FFPE) MM tissue samples.

Design: Proteins were extracted from a micro-dissected FFPE metastatic MM sample and analyzed by 1D SDS-PAGE separation coupled with liquid chromatography-tandem mass spectrometry (GeLC-MS/MS). Acquired MS/MS spectra was submitted for human protein database searching using SEQUEST algorithms, and identified proteins filtered, sorted, and analyzed by bioinformatics software tools. Validatory immunohistochemistry (IHC) was performed on the same FFPE sample.

Results: ~250 µg of protein was extracted from six 10 µm tissue sections of a 0.8 x 0.8 cm tumor sample. 50µg of protein was analyzed by GeLC-MS/MS which identified 930 distinct proteins covering all sub-cellular localizations and a wide variety of biological functions. The false discovery rate was less than 1%. Expression of high abundance proteins was validated by IHC.

Conclusions: GeLC-MS/MS is a valid method to discover and characterize proteins in FFPE MM samples.

499 Immunohistochemical Detection of Merkel Cell Polyomavirus in Kaposi's Sarcoma

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Background: Both Kaposi's sarcoma (KS) and Merkel cell carcinoma (MCC) are more common in the elderly, transplant recipients and AIDS patients. Human herpesvirus 8 (HHV-8) is now acknowledged to be an infective cofactor in KS pathogenesis, whereas a novel polyomavirus (MCPyV) has been identified in MCC. Of interest, MCPyV has also been detected by real-time PCR in 3 of 49 (6.1%) KS cases, although in lower copy numbers than in MCC. Immunohistochemically, mAb CM2B4 detects MCPyV -associated protein with high specificity in most MCC cases. This study was designed to evaluate whether MCPyV can be immunohistochemically detected in KS.

Design: mAb CM2B4 immunostaining was performed on two tissue arrays of paraffin-embedded samples representing 78 KS cases, of which 29 corresponded to classic KS (C-KS) and 49 to AIDS-associated KS (AIDS-KS). mAb CM2B4 was generated against a predicted MCPyV antigenic epitope. Two arrays representative of 34 normal tissues and 36 MCC specimens were used as controls.

Results: Four cases of nodular stage C-KS contained CM2B4-positive cells. The immunoreaction was detected in fewer than 5% of cells and was predominantly faint. In contrast, 70% of MCC cases exhibited CM2B4 positivity in variable cell percentages and with different staining intensities. No normal tissue showed CM2B4-positive staining.

Conclusions: Although unable to provide conclusive evidence in support of MCPyV role in KS pathogenesis, the presence of MCCpV co-infection in four cases of nodal stage C-KS prompts interesting considerations as to its significance. It remains to be elucidated in further studies whether MCPyV is just a bystander for which KS cells provide a suitable proliferation milieu or, conversely, MCPyV infection contributes to local immunosuppression and favors KS development in otherwise healthy elderly patients. The authors wish to acknowledge Yuan Chang, MD for providing us the mAb CM2B4. This study was supported by grant FIS 02/0514.

500 Retained Expression of P- and E-Cadherin Does Not Predict an Epithelial Molecular Phenotype in Melanoma

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Background: Gain-of-function mutations in the small guanine triphosphatase N-Ras and the serine-threonine kinase B-Raf activate the mitogen-activated protein kinase (MAPK) cascade in the majority of melanomas. Development of anti-tumor pharmacotherapy has focused on MAPKs and their effectors for this reason. Yet a recent large-scale RNA expression analysis of 21 human melanoma cell lines identified several with neither Ras nor Raf mutations and no alternate mechanism of MAPK activation (Shields, JM et al. Cancer Res. 67; 4. Feb 15 2007: 1502-12). A unique aspect of these lineages was persistent expression of epithelial markers, including P- and E-cadherin.

Design: To assess both the prevalence of the proposed "epithelial-like" subtype in human melanomas and the potential for P- and E-cadherins to serve as markers thereof, we used immunohistochemistry to determine the level and subcellular locale of P- and E-cadherin, activated (phosphorylated) MAPK, and P-Rex1, a MAPK-dependent regulator of cell migration, in a "progression" series comprising 11 benign nevi, 17 dysplastic nevi with varying degrees of atypia, and 30 melanomas in situ, invasive, and metastatic.

Results: 25 percent of invasive and 22 percent of metastatic melanomas in our series demonstrated an "epithelial-like" phenotype of strong P- and E-cadherin expression paired with minimal MAPK activation, but the former was an unreliable predictor of the latter.

Conclusions: Retained expression of P- and E-cadherin in a significant proportion of the invasive and metastatic melanomas examined is in keeping with recent reports that cadherin expression levels do not always track neatly with melanoma progression. Absence of a correlation between this retained expression and the level of MAPK activity argues for direct assessment of the latter in predicting sensitivity to targeted therapy.

501 Cyclooxygenase-2 in Vulvar Epithelial Neoplasia: Correlation with Invasion and Neutrophil Chemotaxis

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Background: Cyclooxygenase-2 (COX-2) is an inducible regulator of prostaglandin synthesis implicated in tumor proliferation, migration, and angiogenesis. Prior analyses of vulvar epithelial neoplasia have verified its intratumoral expression but provided

conflicting data as to its utility in grading squamous dysplasia or distinguishing in situ from invasive carcinoma.

Design: We collected 147 vulvar epithelial lesions from a regional reference laboratory, nearly doubling the sample size of the largest prior study. We assessed the immunohistochemical staining pattern of COX-2 via the Chalkley point count method, which we deemed a superior means of quantification in light of typically discontinuous expression and the postulated role of the enzyme in potentiating focal microvascular proliferation. In addition, a subjective impression of increased tumor infiltration by neutrophils in cases with high levels of COX-2 prompted us to stain these specimens with anti-neutrophil elastase to assess for a correlation with COX-2 expression.

Results: Point count analysis revealed much higher levels of COX-2 expression in invasive squamous carcinoma versus all non-invasive lesions (mean counts of 9.9, S.D. 7.7, versus 0.6, S.D. 1.8, with $p<0.0001$ by the Welch's t-test). 26 of 31 invasive cases demonstrated significant foci of COX-2 staining versus only 12 of 116 non-invasive cases. Moreover, COX-2 expression in non-invasive lesions was uniformly restricted to basal epithelium overlying dermal papillae; this was distinct from the intraepithelial pattern observed in invasive carcinoma and not sufficiently robust to allow discriminaton between degrees of dysplasia. Staining with anti-neutrophil elastase also revealed a significant correlation between neutrophil aggregates and "hotspots" of intraepithelial COX-2 expression.

Conclusions: Intraepithelial COX-2 expression is a highly specific indicator of invasion in vulvar squamous neoplasia and could prove a useful diagnostic adjunct in superficial or tangential biopsies that complicate assessment of invasive potential. In addition, the correlation of intraepithelial COX-2 expression and intratumoral neutrophil chemotaxis supports a novel feed-forward mechanism for COX-2 upregulation in vulvar squamous carcinoma and suggests that neutrophilic microabscesses may be a histologic hallmark of susceptibility to topical or systemic COX-2 inhibition.

502 Extra-Acral Cutaneous/Soft Tissue Sclerosing Perineuriomas: An Underrecognized Entity in the Differential of CD34 Positive Cutaneous Neoplasms

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Background: Perineuriomas are an uncommon group of tumors composed of perineurial cells of peripheral nerve sheath derivation. They have been further classified as soft tissue, intraneural, sclerosing, and reticular variants; each having distinct immunohistochemical and ultrastructural properties. Sclerosing perineuriomas have been reported to occur almost exclusively on the hands of young men. Histologically the cells are arranged in cords, trabeculae, and chains within a densely sclerotic stroma and the nuclei vary from spindled to round. We describe three examples of cutaneous/soft tissue sclerosing perineuriomas with extra-acral involvement.

Design: We recently encountered three extra-acral cutaneous/soft tissue perineuromas which demonstrated prominent sclerotic change. Each case was evaluated for the amount of sclerotic change, which was further classified as focal or diffuse. Additionally, all three cases were characterized immunohistochemically for expression of EMA, CD34, and S-100.

Results:

Immunohistochemical and histologic findings in extra-acral sclerotic perineuriomas

SITE	AGE	EMA	CD34	S-100	SCLEROSIS	CYTOLOGY
Forearm	46	+ (focal)	+ (diffuse)	-	+ (focal)	Epithelioid/Spindle
Lower lateral leg	60	+ (diffuse)	+ (diffuse)	-	+ (diffuse)	Spindle
Upper lip	84	+ (diffuse)	+ (diffuse)	-	+ (diffuse)	Spindle

Focal >25% to <75%, Diffuse >75%

Conclusions: Recent reports have described the sclerosing variant of perineuriomas as having a predilection for acral regions. Our data demonstrates that perineuriomas with significant sclerotic change also occur in extra-acral locations. Additionally, it is important to recognize that perineuriomas can demonstrate a strong diffuse expression of CD34 and thus should not be confused with other CD34 positive cutaneous spindle to epithelioid cell proliferations. We suggest that when encountering cutaneous spindle to epithelioid cell proliferations that expresses CD34; and extra-acral sclerosing neoplasms, an EMA stain should be considered to aid in the diagnosis of extra-acral sclerotic perineuriomas.

503 Id2 Expression in Cutaneous Nevi and Melanomas

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Background: Inhibitors of DNA binding (Id) proteins are helix-loop-helix (HLH) transcription factors lacking a basic DNA binding domain and hence act as dominant negative factors blocking basic HLH proteins from binding DNA. One member of the Id family, Id2, has been recognized to be overexpressed in some tumors and yet may act as a tumor suppressor in mice. Recently, a role for Id2 in the pathogenesis of melanoma has been proposed because of its decreased expression in an aggressive form of uveal melanoma. This study aims to explore the expression of Id2 in cutaneous benign and malignant melanocytic lesions.

Design: Immunohistochemical staining for Id2 was performed on a tissue microarray of 88 primary malignant melanomas, 20 metastatic malignant melanomas, and 52 benign nevi. Nuclear and cytoplasmic expression of Id2 was scored as a dichotomous variable with "1" defined as no or low expression and "2" defined as moderate to high expression. Data was evaluated and analyzed by Fisher's exact tests and logistic regression.

Results: High nuclear Id2 expression was detected in 96% of benign nevi and 67% of all melanomas ($p<0.01$). The proportion of cases with high nuclear Id2 expression is 72% in primary melanomas and 45% in metastatic melanomas ($p<0.05$). There was also a significant difference in Id2 nuclear expression between the primary melanoma (72%) and the benign nevus (96%) groups ($p<0.01$). Cytoplasmic expression of Id2 was scored as high in 62% of melanoma versus 40% in benign nevi ($p<0.01$). Three risk factors including melanoma tumor thickness, mitotic rate, and presence of ulceration were significantly related to lower nuclear Id2 expression level. No significant association was found between these factors and cytoplasmic Id2 expression.

Conclusions: Nuclear Id2 expression significantly decreases from benign nevi to primary melanomas and metastatic melanomas. In addition, decreased nuclear Id2 expression seems to correlate with tumor progression demonstrated by the inverse correlation of nuclear expression of Id2 with tumor thickness, mitotic rate, and the presence of ulceration. Loss of nuclear Id2 expression may play an important role in cutaneous melanoma tumorigenesis.

504 Increased Cyclin D1 (CCND1) Protein Is Associated with Reduced MIR-193B Levels in Melanoma: A Potential Melanoma Regulatory Pathway

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Background: Cutaneous melanoma is an aggressive form of human skin cancer characterized by high metastatic potential and poor prognosis. Emerging evidence suggests that microRNA dysregulation may play a role in melanoma development. In our initial study, we found miR-193b is downregulated in malignant melanomas relative to benign nevi. Furthermore, our functional studies demonstrated that miR-193b might regulate cell proliferation by downregulating expression of cyclin D1 in melanoma cell lines.

Design: We speculated that the downregulation of miR-193b level might contribute to the upregulation of cyclin D1 in melanoma. To confirm this, we assessed cyclin D1 level on a TMA comprised of 8 benign nevi, 7 primary melanomas and 8 metastatic melanomas using immunohistochemical staining. The scanned TMA image was scored for intensity of staining by Aperio's Immunohistochemistry image analysis. The miR-193b levels in those melanocytic tissues were examined in our previous microarray study. We compared the miR-193b expression levels to the percentage of positive nuclei for cyclin D1.

Results: The percentage of positive nuclei of staining for Cyclin D1 is upregulated from benign nevi (9.53 ± 3.62%) to primary melanomas (24.22±9.47%) and metastatic melanomas (37.88±7.85%) while miR-193b expression is downregulated from benign nevi (583.08±50.66) to primary melanomas (230.55±75.31) and metastatic melanomas (160.60±36.11). Regression analysis confirmed a weak negative correlation ($R = -0.45$).

Conclusions: Our TMA validation study appears to support our hypothesis that miR-193b repress cyclin D1. Because miR-193b appears to have anti-proliferative effects in melanoma cells, it may have potential as a novel diagnostic and therapeutic tool. Our future direction will be to examine a larger melanoma sample set to test the correlation of miR-193b and cyclin D1.

505 Immunohistochemical Profile Distinguishes Spitz Nevi from Melanomas

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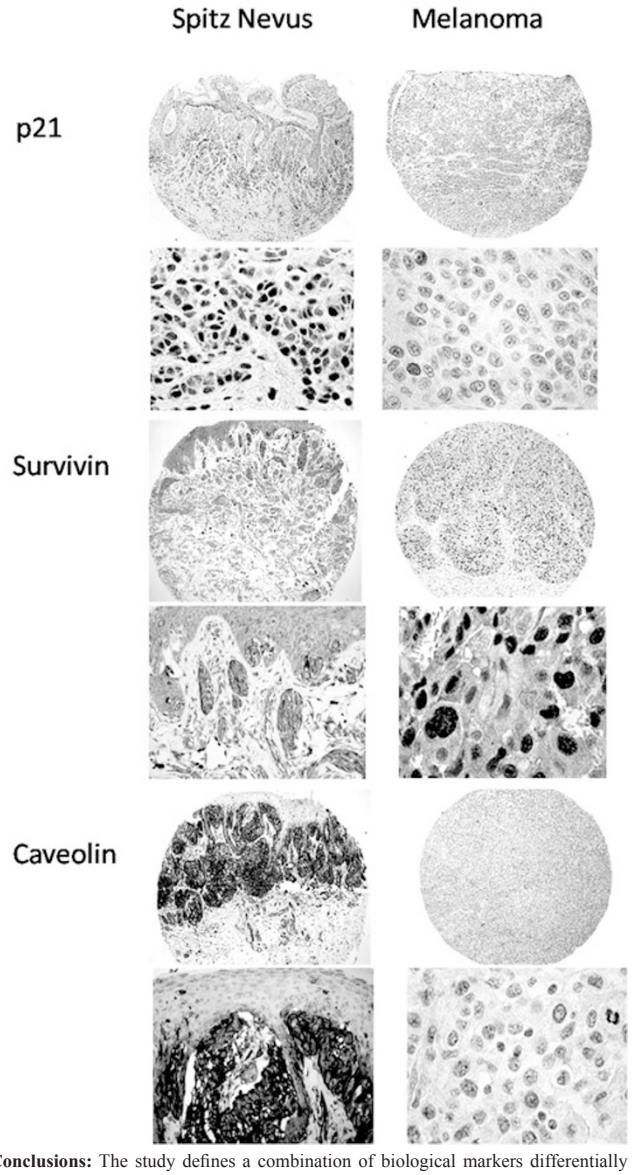
Background: Several isolated markers have been proposed to aid in differential diagnostic of difficult melanocytic lesions, albeit none has been demonstrated definitive in differentiating Spitz nevus from melanoma.

Design: This study proposes a wide panel of 22 markers playing key roles in different biological functions including cell cycle regulators, apoptotic markers, DNA repair proteins and membranous receptors in order to provide a combination of proteins associated with either benign or malignant phenotype. Using tissue microarrays, we compared protein expression profiles in 28 typical Spitz nevi and 62 primary vertical growth phase non-spitzoid melanomas.

Results: Most of the significant differences were linked to cell cycle deregulation such as over-expression of cyclin D1 and p21 in Spitz nevi compared to non-spitzoid melanomas (74% *vs.* 16%: $p<0.005$ and 91% *vs.* 27%; $p<0.005$ respectively) and mitotic rate including Ki-67, highly expressed in deep areas of non spitzoid melanomas (37%) whereas Spitz nevi didn't express it (0%: $p<0.005$), topoisomerase IIα (79% in non-spitzoid melanomas *versus* 15% in Spitz nevi, $p<0.005$) and nuclear survivin (69% in melanomas *versus* 0% in Spitz nevi, $p<0.005$). In addition, osteonectin (SPARC) and protein kinase Cα (PKCα), previously well recognized to have an important role in local invasion, have shown to be significantly overexpressed in Spitz nevi (96% for both markers) compared to melanomas (43% and 48% respectively).



Conclusions: The study defines a combination of biological markers differentially expressed in Spitz nevi from non-spitzoid melanomas providing a potential tool for histopathological differential diagnosis between Spitz nevus and melanoma. Nevertheless, more studies including atypical Spitz nevi and spitzoid melanomas are necessary to further establish a reliable panel to differentiate among difficult cases.

506 A Mouse Model of Melanoma Driven by Oncogenic KRAS

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Background: Hyper-activation of RAS/RAF/MEK/ERK pathway is present in 30% of malignant tumours and may occur due to activation of several of its members. In melanomas, NRAS is mutated in 19% of cases, whereas HRAS and KRAS are mutated in 5 and 2% respectively. Previous animal models have shown that activation of ^{G12V}HRAS and ^{Q61K}NRAS are able to induce melanoma formation. Here we describe a mouse model of melanoma driven by ^{G12V}KRAS.

Design: We used a Cre recombinase/*loxP* system in which ^{G12V}KRAS expression is induced in the melanocytic lineage following topical application of tamoxifen. Selected lesions were subjected to immunohistochemical analysis with antibodies against S100, Ki67 and p16 proteins, and reverse transcriptase PCR (RT-PCR) for Tyrosinase, Trp2, Pax3, Silver.

Results: The mice developed skin hyper-pigmentation and skin lesions of varying pathological features and degree of malignancy as early as one month after tamoxifen application. Dermal lesions were present in 30% of the mice, resembled human blue naevi, were composed of pigmented epithelioid and dendritic cells, and expressed very low levels of Ki67 (<1%). In peri-orbital areas, slightly larger lesions, consistent with the diagnosis of pigmented epithelioid melanocytoma were observed,. Furthermore, rapidly-growing lesions, predominantly composed of hypo/amelanotic, mitotically active, spindle cells, developed in the back and peri-anal areas of 80% of the treated mice. These lesions displayed aggressive behaviour, invading and destroying the subcutaneous tissue and skeletal muscle. Diffuse positivity for S100 and expression of melanoma markers by RT-PCR confirmed the diagnosis of malignant melanoma. Additionally, cultured cells from these lesions were able to seed to the lungs of nude mice when injected via the tail vein.

Conclusions: Expression of oncogenic KRAS in melanocytes can induce naevus formation and melanomagenesis *in vivo*. Establishing an *in vivo* model of KRAS-driven melanoma provides novel opportunities to examine the apparent differences between the three RAS family members and to develop therapeutic approaches tailored to melanomas driven by mutation of specific RAS genes.

507 Type of Mammary Paget Disease Is Associated with Disease Free Survival

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Background: Mammary Paget disease (MPD) is a relatively rare entity with a heterogeneous clinical presentation. Previous reports on this tumor have focused on their differential diagnosis and histogenesis on a limited population. This retrospective study was designed to evaluate the clinicopathologic features and outcomes of a cohort of patients diagnosed with MPD and seen at a single institution.

Design: A search for Paget in the nipple or skin of breast recovered 546 patients between 1985 and 2009. Pathology features, clinical presentation, radiologic findings, recurrence rates, and disease-free (DFS) and overall survival (OS) were available in 321 cases. At the time of submission information on all variables was obtained on 173 patients.

Results: Three groups of MPD patients were observed: 15 Paget without underlying breast carcinoma (PD), 67 Paget with DCIS (PDCIS), and 91 Paget with invasive cancer (PInv). The mean age at diagnosis for MPD was 54 yrs (range 21 to 90 yrs). Clinically, nipple discharge was present in 13% to 28% and nipple inversion was present in 0% to 12% of MPD. Skin changes were present in 60% of PD, 58% of PDCIS and 35% of PInv. Abnormal radiologic findings were found in 50% of PD, 81% of PDCIS and 90% of PInv. Palpable tumor was present in 9% of PD, 25% of PDCIS and 60% of PInv. Hormone receptor was positive in 51% to 54% in PDCIS and PInv. In tumors associated with MPD, HER2 was overexpressed in 58% of PInv and 67% of PDCIS. In Paget cells, HER2 was expressed in 50% of PD, 75% of PDCIS, and 64% of PInv. Coexpression of HER2 in both Paget cells and carcinoma was seen in 40% of PInv and 60% of PDCIS. There was no significant difference in OS within MPD. The median DFS was different among the three groups. Only 1 of 15 PD recurred. The median DFS was 9.9 yrs for PDCIS and 4.5 yrs for PInv. Further studies are required due to the small sample size of PD.

Conclusions: Skin changes were more likely to be present in PD and PDCIS as compared to PInv. However, abnormal radiologic findings and palpable tumor were most frequently found in PInv. The Paget cells in MPD expressed HER2 in the majority of cases. Moreover, HER2 was more likely to be positive in the nipple as compared to the underlying carcinoma. Although, OS was not significantly different within MPD, median DFS in PInv was shorter than PDCIS and PD. In conclusion, our results suggest that the types of MPD have different clinicopathologic characteristics and disease free survival.

508 CD163: A Highly Specific Marker for Myeloid Leukemia Cutis

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Background: Recognition of myeloid leukemia cutis (LC) is clinically important. However, currently used immunohistochemical markers can be insufficiently sensitive and/or specific for definitive diagnosis. CD163, a haptoglobin-hemoglobin scavenger receptor, has been shown to be largely restricted to the monocyte/histiocyte lineage. We investigated the utility of CD163 in the diagnosis of myeloid LC with a monocytic component.

Design: A total of 34 cases, including 18 cases of myelomonocytic or monocytic LC (acute myeloid leukemia, M4 or M5), 10 cases of myeloid LC without monocytic component, and 6 cases of T- or B-cell acute lymphoblastic leukemia/lymphoma, were stained for CD163.

Results: We observed CD163 expression in 8 of 18 (44%) of myelomonocytic or monocytic LC and 1 of 10 (10%) of other myeloid LC. CD163 was not expressed in any of the acute lymphoblastic leukemia/lymphoma cases (0/6). Results are summarized in Table 1.

Table 1. CD163 expression by immunohistochemistry in leukemia cutis.

Diagnosis	CD163
AML-M4	4/11 (36%)
AML-M5	3/6 (50%)
AML-M4-5	8/18 (44%)
AML, other than M4-5	1/10 (10%)
AML, all	9/28 (32%)
ALL	0/6 (0%)

Numbers of positive cases are given as a fraction over total cases stained. Abbreviations: AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia/lymphoma

Conclusions: These results suggest that, in combination with currently used immunohistochemical markers, CD163 is valuable as a specific marker for myeloid LC with monocytic differentiation.

509 CD25 Expression in Cutaneous B-Cell Lymphomas

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Background: CD25, the alpha subunit of interleukin receptor-2, is expressed on activated T-cells, B-cells, and monocytes. CD25 expression is observed in some systemic and cutaneous T-cell malignancies. Denileukin diftitox (Ontak), an agent targeting CD25, has been approved for use in T-cell lymphomas, and has demonstrated activity against some B-cell lymphomas. CD25 reactivity in B-cell malignancies is not well understood and, to our knowledge, its expression in cutaneous B-cell neoplasms has not been explored.

Design: We conducted immunohistochemical staining for CD25 expression in 6 cases of cutaneous benign lymphoid hyperplasia (LH), 7 primary cutaneous low-grade B-cell lymphomas (C-BCL), 9 cutaneous diffuse large cell B-cell lymphomas (DLBCL)-leg type, 6 primary cutaneous DLBCL-other, 5 systemic B-cell lymphomas (S-BCL) with secondary skin involvement, and 6 systemic DLBCLs with skin involvement. CD25 positivity was defined as at least 10% of lesional cells expressing CD25.
Results: Among primary cutaneous B-cell lymphomas, CD25 stained 5/9 DLBCL-leg type and 1/6 DLBCL-other (which was a case of EBV+ angioinvasive DLBCL). Two systemic DLBCLs (2/6) were positive for CD25. All cases of lymphoid hyperplasia, C-BCL, and S-BCL displayed scattered CD25+ small lymphocytes (likely activated T cells), which comprised less than 10% of infiltrating cells.
Conclusions: 1. CD25 expression was more frequent in cutaneous DLBCL-leg type relative to cutaneous DLBCL-other. 2. CD25 reactivity may be observed in both cutaneous DLBCL-leg type and systemic DLBCL with skin involvement, and cannot be used to distinguish the two. 3. No significant CD25 expression was found in cutaneous low-grade B-cell lymphomas or benign lymphoid hyperplasia in this small study. 4. CD25 may serve as a potential therapeutic target in cutaneous DLBCL-leg type.

510 Therapeutic Potential of Increased Intratumoral Cytotoxic CD8+T Cells Following Antibody-Induced CTLA4 Blockade in Metastatic Melanoma

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Background: Blockade of CTLA4 (a major inhibitor of anti-cancer cellular immunity) induces persistent responses in ~10% of patients with metastatic melanoma. Though not prospectively demonstrated in humans, preclinical models predict that CTLA4 blockade will increase T cell accumulation in tumors. We quantified immune infiltrates in biopsies of patients treated with the fully human anti-CTLA4 monoclonal-antibody tremelimumab (Pfizer Inc).
Design: Pre- and post-treatment biopsies (pre-bx and post-bx) from 20 patients with advanced melanoma (SIIIc-M1) treated with tremelimumab (15 mg/kg q3 months) were evaluated for density of intratumoral infiltrates (ITI) and peritumoral infiltrates (PTI) by $CD4^+$ThLs and $CD8^+$CTLs using IHC and computer-based imaging system analyses.
Results: $CD8^+$CTL ITI was 955.06±621.08/mm^2 in post-bx vs. 296.12±278.85 in pre-bx (mean increase: 658.94, P=0.0006). $CD4^+$ThLs ITI post-bx was 305.52±116.20/mm^2 vs. 116.20± 141.52 pre-bx (mean increase 189.31, P=0.0004). $CD8^+$CTLs PTI was 1099.81±1034.45/mm^2 in post-bx vs. 568.57±513.86 in pre-bx (mean increase: 531.23; P=0.1309). $CD4^+$ThL PTI was 777.74±694.96/mm^2 post-bx vs. 439.64±377.46 pre-bx (mean increase: 338.09; P=0.2661). CD8 infiltration and associated regression of melanoma paralleled clinical responses. 3 patients had a durable complete clinical response after treatment of tremelimumab: 1 with rare individual melanoma cells left swamped by $CD8^+$CTL (post vs. pre: 958.19 vs. 140.48) with concomitant increase in $CD4^+$ThL (1142.27 vs.142.19) infiltration; 1 with very dense T cell infiltration (ITI-post vs. pre: $CD8^+$CTLs 955.79 vs. 77.95; $CD4^+$ThL 545.65 vs. 0.00); another patient had a delayed biopsy that showed fibrotic replacement of a regressed melanoma. Patients without clinical responses also had marked increases of ITI density in post-bx after treatment: in 8 patients the increase of $CD8^+$CTLs density was greater than the average increase (658.94); 6 patients had an increase of $CD4^+$ThLs density greater than average (189.31).
Conclusions: The CTLA4 blocking antibody tremelimumab significantly increases intratumoral infiltration by $CD8^+$CTL and $CD4^+$ThL, demonstrating markedly enhanced immune infiltration of tumors in most patients. This provides direct evidence of one component of the mechanism by which this antibody induces durable tumor responses in metastatic melanoma patients. Since some patients have increased CTL, but do not respond clinically, there are clearly other factors involved that remain to be explored.

511 Molecular Detection of Circulating Sezary Cells in Patients with Mycosis Fungoides

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Background: Mycosis fungoides (MF) is a primary cutaneous T-cell lymphoma and prognosis depends on clinical stage. While patients with disease confined to the skin have an excellent prognosis, advanced stage, Sezary Syndrome (SS) particularly, carries a worse outcome. We investigate circulating Sezary cells in MF patients by using PCR based T-cell receptor (TCR) gene rearrangement studies, in addition to flow cytometry and morphologic examination.
Design: 104 patients were identified with clinical findings/skin biopsy consistent with MF. All had flow cytometric immunophenotyping of peripheral blood. 96 cases had TCR gene rearrangement studies on peripheral blood; 78 had TCR analysis on skin biopsy. Retrospective analysis was performed and the rate of positivity for each test was determined. Clonal PCR products were compared in cases with positive PCR in both peripheral blood and skin to determine the rate of true positivity (positive predictive value).
Results: Of 104 cases, 16 (15.4%) showed circulating Sezary cells by flow cytometry. Of 83 cases with negative flow cytometry and TCR gene rearrangement performed on peripheral blood, 22 (26.5%) showed clonal rearrangements. 74 cases had TCR gene rearrangement studies on both peripheral blood and skin. Of these, 15 cases had clonal peaks in both peripheral blood and skin. Of those 15 cases, 7 were positive by flow cytometry and had matching base pair length of clonal peaks in the peripheral blood and skin. Of the remaining 8 cases with negative flow cytometry, 5 (62.5%) demonstrated matching clonal peaks in the peripheral blood and skin. 3 of those 8 cases (37.5%) showed different clonal peaks, likely representing false positives. Given a 62.5% positive predictive value for 26.5% of cases (22/83), the matching rate for clonal PCR products in the peripheral blood and skin is projected to be 16.56% among 83 cases with negative flow cytometry.
Conclusions: Of cases with no detectable circulating Sezary cells, 26.5% show clonal TCR gene rearrangement in peripheral blood. The positive predictive value for this is 62.5% based on matching base pair length of PCR products in blood and skin; thus, the projected rate of matching clonal products is 16.5%. This may represent subclincal circulating Sezary cells. 2 cases had follow up flow cytometry, 1 of which demonstrated circulating Sezary cells 7 months later and subsequently developed SS. More follow up is needed to determine the significance of a subclinical circulating clone and the utility of molecular monitoring for early identification of disease progression.

512 Flat Pleomorphic Fibromas: Report of Three Cases of an Unusual Morphologic Variant of Pleomorphic Fibroma

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Background: Pleomorphic fibromas of the skin are rare benign dermal neoplasms composed of spindle and stellate cells admixed with haphazardly arranged thickened collagen bundles. Some of these cells can be markedly atypical, exhibiting nuclear pleomorphism, hyperchromasia and occasional multinucleation. However, mitotic figures are exceedingly rare.
Design: In this report we describe three cases of an unusual morphologic variant of pleomorphic fibroma.
Results: All three patients were middle-aged women who presented with predominantly flat dermal lesions with an infiltrative growth pattern, in contrast to the typically reported polypoid or dome-shaped lesions. In two of the cases the unusual histological presentation was coupled with the presence of prominent cellular pleomorphism and deep nodular lymphocytic infiltrates, raising the specter of desmoplastic melanoma. However, the discrepancy between the marked cellular atypia and the lack of prominent mitotic figures, as well as the absence of any other morphological findings suggestive of melanoma were helpful hints in the diagnosis.
Conclusions: Recognizing unusual flat variants of pleomorphic fibroma from infiltrative cutaneous malignant neoplasms is of utmost importance and has major implications for treatment and prognosis. Pleomorphic fibromas are benign in behavior and complete excision is usually curative. Local recurrences are rare and systemic spread has not been reported. Immunohistochemical studies have limited utility in recognizing these unusual variants and careful examination of the morphologic pattern remains the gold standard for diagnosis.

513 Expression Analysis of Homing Molecules in Cutaneous and Systemic Mastocytosis

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Background: Mastocytosis is a rare disorder characterized by accumulation of mast cells in one or more organs. Cutaneous mastocytosis can present as urticaria pigmentosa or mastocytoma. In children, this disease has a benign course, whereas in adults there is a chance of having associated systemic disease. In systemic mastocytosis, mast cells can be found accumulated in various tissues, most often skin and bone marrow, but also liver, spleen, and the gastrointestinal tract. Involvement of homing molecules/receptors, adhesion molecules, and chemokines in lymphocyte trafficking has been described in cutaneous inflammatory disorders and lymphomas. Currently, not much is known about the mechanisms involved in the recruitment and accumulation of neoplastic mast cells in specific organs as in mastocytosis.
Design: We used immunohistochemistry to investigate the potential role of homing molecules in mast cell recruitment to the skin and other sites in mastocytosis. Skin biopsies from thirty-two patients with cutaneous mastocytosis and two cases of systemic mastocytosis, liver and bone marrow, confirmed by CD117 or mast cell tryptase (MCT) were stained. Immunohistochemistry was performed with antibodies directed against various homing molecules, including CD29 (β1-integrin), CD54 (ICAM-1), CD62-L (L-selectin), CD183 (CXCR3), sialyl-Lewis x (SLX).
Results: CD183 was expressed in mast cells in 30 of 32 cutaneous cases, CD29 was expressed in 28 of 32 cutaneous cases, while CD54, CD62L, and SLX were consistently negative in cutaneous mast cells. CD54 was negative in mast cells; however, the dermal vessels consistently stained positive for CD54. In the systemic cases of mastocytosis, the liver mast cells expressed CD29 but not CD183 and bone marrow mast cells expressed CD183 and CD29. CD54 was negative in both systemic cases.
Conclusions: In summary, we have demonstrated in this study that accumulated mast cells in cutaneous mastocytosis express CD183 and CD29, and lack expression of CD54, CD62-L and sialyl Lewis x. Thus, unlike cutaneous lymphomas or leukemia cutis, cutaneous mastocytosis does not appear to involve selectins, but integrins and chemokines may be important for mast cell accumulation in skin. Additional studies are in progress, including testing ligands of molecules presented in this report, and comparison with additional bone marrow lesions in systemic mastocytosis.

514 Fibroblastic Rheumatism: A Report of Three Cases

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Background: Fibroblastic rheumatism is a rare dermatoarthropathy characterized by the sudden onset of cutaneous nodules, flexion contractures and polyarthritis that may progress to destructive arthropathy. The cutaneous manifestations can present concurrently, precede or follow development of arthropathy. Histopathology, in the correct clinical context, confirms the diagnosis.
Design: Cases coded as fibroblastic rheumatism were retrieved from institutional and consultation files (SDB). Charts and biopsies were reviewed. In selected cases, elastic stains and immunostains for SMA, S100, CD34, desmin and EMA were performed.
Results: Three cases were identified. A 6-year-old Caucasian boy presented with a several month history of arthralgias and cutaneous nodules on the scalp and extremities

that developed approximately six weeks after an upper respiratory tract infection. A 15-year old Filipino boy presented with cutaneous nodules on the hands and developed flexion contractures; he subsequently developed nodules on the elbows, feet and face. A 60-year-old Puerto Rican man presented with tender cutaneous nodules on the scalp, face and extremities. He had a history of inflammatory arthritis 2.5 years prior involving the wrists, fingers, elbows, shoulders, knees and ankles. In the pediatric cases, the biopsies demonstrated fascicles of bland spindled cells within the dermis that resembled early scar or fibromatosis. In the adult case the lesions were less cellular with more randomly arranged fibroblasts in a densely collagenous stroma. The nuclei were bland without hyperchromasia or mitotic activity. Adnexal structures in the dermis were relatively spared. Elastic fibers were absent in the fibroblastic proliferation (2 of 2). Uninvolved portions of the dermis had normal appearing elastic fibers. SMA stains highlighted the lesional cells in a myofibroblastic pattern in 1 of 2 cases. No immunoreactivity was seen for S100 (n=2), CD34 (n=1), desmin (n=1) or EMA (n=1). The overlying epidermis was unremarkable. The 6-year-old boy has responded to methotrexate. Methotrexate was contraindicated in the adult and refused by the 15-year-old.

Conclusions: Fibrobolastic rheumatism is a rare dermatoarthropathy characterized by multiple scar-like or fibromatosis-like nodules and arthralgias. Loss of elastic fibers in the fibroblastic proliferation is characteristic. Presentation as multiple lesions is an important clue to the diagnosis. Correlation with clinical history is critical to avoid misdiagnosis as other entities such as fibromatosis or scar.

515 Reticulin and NM23 Staining in the Interpretation of Lymph Nodal Nevus Rests

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Background: Melanocytic nevus rests in lymph nodes are a known diagnostic challenge, especially in patients with a history of melanoma. Reticulin and NM23 have been studied in melanocytic lesions of the skin. The pattern of reticulin staining in melanomas surrounds groups/nests of melanocytes but individual cells in benign nevi. NM23, a metastasis-suppressor gene, has an association with metastatic potential in melanomas and some carcinomas. We sought to evaluate the differential diagnostic utility of these markers in capsular nevus rests (CNR) and malignant melanoma (MM) metastatic to lymph nodes.

Design: Archival paraffin-embedded lymph nodes of 28 cases from three academic centers (14 cases of MM to lymph nodes and 14 cases of CNR, all confirmed with Melan-A staining) were stained with reticulin (DAKO kit), and NM23 (1:3000, monoclonal, Santa Cruz). In 27 cases, the patient had a history of melanoma, and in 1 the patient had a history of breast cancer. The pattern of reticulin staining was reported as surrounding groups if staining was noted around 5-10 melanocytes in greater than 50% of the lesion, but was otherwise reported as surrounding individual melanocytes. Strong cytoplasmic staining was considered to represent reactivity for NM23.

Results: Reticulin staining around groups of melanocytes was identified in all 14 cases of MM. In the majority of cases, staining was noted around large nests (>20 melanocytes). Regarding CNR cases, 12 of 14 cases (79%) demonstrated staining around individual melanocytes, while in 2 cases, reticulin surrounded melanocytic groups. NM23 staining was equivocal in all cases of MM and CNRs, with adequate labeling of controls.

Conclusions: Reticulin staining reliably invests groups of melanocytes in cases of metastatic melanoma, while in capsular nevus rests, it predominantly surrounds individual melanocytes. NM23 demonstated no discriminatory value in this analysis, in contrast to the results of prior reports. In cases in which a collection of melanocytes is present within a lymph node, reticulin deposition around individual melanocytes supports a diagnosis of capsular nevus rest.

516 Benign and Malignant Neoplasms in a Series of 85 Patients with Brooke-Spiegler Syndrome, Including the Phenotype of Multiple Familial Trichoepitheliomas

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Background: Brooke-Spiegler syndrome (BSS) is an inherited autosomal dominant disease characterized by the development of multiple adnexal cutaneous neoplasms including spiradenoma, cylindroma, spiradenocylindroma and trichoepithelioma. Multiple familial trichoepitheliomas (MFT) are regarded as a variant of BSS due to the overlap in the clinical phenotype of the two conditions and common underlying genetic abnormalities (mutations in the CYLD gene). Cylindroma is usually cited as the most common benign tumor in BSS. Malignant neoplasms are rare.

Design: To investigate the morphological spectrum of benign and malignant neoplasms we studied a series of 85 patients affected with BSS (female 57, male 28) ranging in age from 11 to 81 years (median 50 years), including 70 patients with the classical BSS phenotype and 15 patients with MFT.

Results: A total of 446 benign tumors available for review included 133 trichoepitheliomas, 118 spiradenomas, 110 spiradenocylindromas, 81 cylindromas, 3 small nodular trichoblastomas, and 1 lymphadenoma. One patient had a cylindroma involving the breast parenchyma. Malignant neoplasms were identified in 9 patients, of whom 8 presented with classical BSS and 1 individual with MFT. The malignant neoplasms included 6 carcinomas bearing a resemblance to basal call adenocarcinoma of salivary glands, either low grade or high grade, all of which were associated with a preexisting spiradenoma or cylindroma. In the remaining 3 patients, the malignant lesions were basal cell carcinoma, including large nodular, small nodular, and infundibulocystic variants.

Conclusions: Spiradenoma is the most common benign neoplasm in patients with the classical phenotype of BSS, whereas cylindroma is the rarest one. Malignant lesions appear to be more common in BSS patients with the classical phenotype compared to MFT.

517 Differential Gene Expression of Primary Melanoma Versus Sentinel Lymph Node Metastasis

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Background: Melanoma is a potentially fatal cutaneous neoplasm with no effective treatment and 48,000 yearly deaths worldwide (WHO). Understanding of molecular mechanisms of metastasis is limited, contributing to the absence of effective treatments. Altered expression of genes that underpin molecular events leading to metastasis is of particular interest. We investigated gene expression profiles of primary melanomas and melanomas metastatic to sentinel nodes by DNA microarray technique.

Design: 8 primary melanomas and 3 melanomas metastatic to sentinel node were retrieved from UCLA archives. 2-4mm lesions were dissected from formalin fixed, paraffin-embedded blocks. Total mRNA was isolated, amplified and labeled using anAmbion Recover All™ Total Nucleic Acid Isolation kit, Nu-GEN WT-Ovation® FFPE RNA Amplification System and FL-Ovation® cDNA Biotin Module V2, respectively. Samples were then hybridized to the Affymetrix Gene Chip® Human U133 Plus 2.0 Array. Data analyses were performed using Partek® Genomics Suite Version 6.4. Differentially expressed genes were selected at >=2fold and $p<0.05$.

Results: Hierarchical clustering of the melanocytic lesions disclosed two distinct groups: the 8 primary melanomas and 3 sentinel node metastases. Analysis identified 278 statistically significant genes that were differentially expressed. Most differences were associated with decreased expression of genes by sentinel node metastases relative to primary melanomas. The reverse was rare. Interesting genes with relatively decreased expression in the sentinel node tumors were gap junction protein, keratin genes and stratifin.

Conclusions: DNA microarray system showed striking differential gene expression patterns between primary and nodally metastatic melanomas. From our list of significant genes, there is a predominant trend of relatively decreased expression of genes in nodal metastases relative to primary melanomas. Down regulation of genes is globally correlated with nodal metastasis by melanoma. Decreased genes are critically involved in cellular structural integrity (gap junction protein, keratins) and also in tumor suppression (stratifin). These initial studies of melanoma show that critical genes are differentially expressed in primary and nodal metastasis: preliminary findings that are consistent with current understanding of the molecular biology of tumors and may explain molecular events that underlie melanoma metastases and lead to the development of novel effective therapy.

518 Merkel Cell Carcinoma Immunoreactivity with PAX-5 and TdT – A Potential Diagnostic Pitfall?

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Background: Merkel cell carcinoma (MCC) is a high-grade neuroendocrine carcinoma of skin characterized by cells with a "blastic" appearance, scant cytoplasm, and fine, evenly distributed chromatin. It can sometimes be difficult to accurately diagnose MCC by conventional light microscopy due to its histologic similarity to other "small round blue cell tumors". Ancillary techniques particularly immunohistochemistry are usually required to make a definitive diagnosis. Terminal deoxynucleotidyl transferase (TdT) is a DNA polymerase present in thymic T cells, lymphoblastic leukemia/lymphoma, and some cases of acute myeloid leukemia. PAX-5 is a B cell specific transcription factor crucial for B-cell ontogeny and has been detected in most human B-cell lymphomas. We recently encountered a case of MCC which expressed PAX-5 and TdT resulting in its initial misinterpretation as B-lymphoblastic leukemia/ lymphoma. The aim of our study was to evaluate the expression of PAX-5 and TdT, markers of B-lymphoblastic leukemia/lymphoma (B-ALL), in a cohort of patients with MCC and determine if their expression was a consistent finding in these tumors.

Design: We retrieved 7 cases with initial diagnosis of MCC from our institution. Archival blocks and slides were retrieved and reviewed and clinical information obtained from patient charts.The diagnosis of MCC was confirmed in all cases. These 7 cases were stained with PAX-5, TdT, cytokeratin, synaptophysin, chromogranin, CK20 and TTF1. Immunohistochemical staining was scored as: negative (-), weak (1+), moderate (2+) or strong (3+).

Results: Immunohistochemical positivity was as follows: PAX-5 (7/7), TdT (4/7, 57%), PAX-5 and TdT co-expression 4/7 (57%), Cytokeratin (7/7) (both membrane and perinuclear dot positivity), Synaptophysin (7/7), Chromogranin A (7/7), and TTF-1 (0/7). The PAX-5 staining was strong in 6/7 (86%) and weak in 1/7 (14%) cases. TdT staining was strong in 2/7 (29%), moderate in 1/7 (14%) weak in 1/7 (14%) and negative in 3/7 (43%) cases.

Conclusions: Co-expression of TdT and PAX-5 by MCC may result in its misdiagnosis as B-ALL particularly since the latter is often negative for CD45, a frequently used lymphoid marker. Pathologists should be aware of this diagnostic pitfall so as to avoid misinterpretation of immunohistochemical results as evidence of lymphoma, particularly in small biopsies. PAX-5 and TdT positivity in conjunction with neuroendocrine markers further support the diagnosis of MCC when evaluating cutaneous small round blue cell tumors.

519 CD1a+ Cutaneous Dendritic Cells, Langerhans Cells and Interleukin 3 Receptor (CD123) in Reactive and Neoplastic Cutaneous Lymphoid Proliferations

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Background: Distinguishing reactive cutaneous lymphoid infiltrates from neoplastic proliferations can be difficult. Cutaneous CD1a expressing dendritic cells (CDCs) and Langerhans cells (LHCs) play crucial role in skin immune responses and T-cell proliferation. Interleukin-3 (IL-3) secreted by T cells promotes proliferation and differentiation of dendritic cells. We examined distribution of CD1a+ CDCs, LHCs and expression of IL-3 receptor (IL-3R; CD123) in a variety of reactive, neoplastic and pseudoneoplastic cutaneous lesions.

Design: 55 skin biopsies were retrieved form pathology archives. The diagnostic categories included: arthropod bite (13), Langerhans cell histiocytosis (4), juvenile xanthogranuloma (JXG;15), reticulohistiocytoma (RH;3), B-cell lymphoma (5), CD30+ T-cell lymphoma (2), mycosis fungoides (MF;2), lymphomatoid papulosis (LYP;1), atypical T-cell infiltrate (1), peripheral T-cell lymphoma (1) and angiolymphoid hyperplasia with eosinophilia (ALHE; 8). Immunoperoxidase staining for CD1a and IL-3R (CD123) was performed with commercially available antibodies. Quantity, distribution, morphology and staining intensity of CD1a+ CDCs, LHCs and cells expressing CD123 were recorded.

Results: All arthropod bites showed transdermal perivascular CD1a+ CDCs with mature morphology and prominent CD123+ cells in association with perivascular lymphoid infiltrates. In T-cell lymphomas (4/5), LYP, and atypical T-cell infiltrate CD1a+ CDCs were absent or infrequent. One case of MF showed prominent CD1a+ CDCs in the dermal papillae and associated prominent LHCs. CD123+ cells were prominent in the dermis in LYP, atypical T-cell infiltrate, and one case of CD30+ T-cell lymphoma. A subset of neoplastic cells in one CD30+ T-cell lymphoma case was positive for CD123. Most JXG (14/15) and RH (2/3) did not contain significant CD1a+ CDCs or CD123+ cells. ALHE demonstrated variable CD1a+ CDCs and CD123+ cells. In Langerhans cell histiocytosis neoplastic cells showed immature phenotype with membranous and cytoplasmic CD1a staining. Associated CD123+ cells were single and scattered. In B-cell lymphomas CD1a+ CDCs and CD123+ cells were rare.

Conclusions: IL-3R (CD123) plays a possible pathogenetic role in a subset of cutaneous T-cell neoplasms. It can be a useful tool in evaluation of atypical cutaneous lymphoid infiltrates, especially when combined with CD1a. Prominent perivascular intradermal CD1a+ CDCs, particularly in association with CD123 positive cells, favor reactive process.

520 An Analysis of Prognostic Markers in Basal Cell Carcinoma: Can We Predict Behavior?

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Background: Basal cell carcinoma (BCC) is a neoplasm with an indolent clinical course; however, rare cases behave in an aggressive fashion. While long duration, large size, and an infiltrative pattern have been associated with an increased risk for metastasis, the correlations are too weak to have clinical significance. Currently there are no reliable clinical, histopathologic, or immunohistochemical features that can distinguish aggressive from nonaggressive BCCs. This prompted us to evaluate the utility of several morphologic and immunophenotypic parameters as prognostic markers in BCC.

Design: We retrieved from our clinical database 10 cases of clinically aggressive BCC characterized by metastasis (1 case), large size >11cm (2 cases), aggressive local invasion (3 cases), or recurrence (5 cases). In addition we selected a control group of 56 BCCs (25 nodular, 3 micronodular, 23 infiltrative, 4 metatypical, 1 morpheaform). All cases were reviewed for the following parameters: histologic pattern, mitotic index (mitoses/mm2), apoptosis, necrosis, squamous differentiation, neuroendocrine morphology, (defined as high nuclear to cytoplasmic ratio, nuclear molding, homogeneous chromatin pattern, inconspicuous nucleoli) peripheral palisading, and ulceration. Immunohistochemistry for CK20, synaptophysin, and chromogranin was also performed. Percent of tumor cells staining as well as a staining intensity score was recorded for each case. Staining was considered positive when greater than 10% of cells stained.

Results: The only parameter that showed a significant difference in aggressive BCC vs. the control group was presence of necrosis 8/10 (80%) vs. 9/56 (16%), respectively, $p<0.001$. There were no differences in age distribution of patients with aggressive vs. nonaggressive BCC. Additionally, there was no difference in the number of mitoses, presence or absence of apoptosis, squamous differentiation, nuclear molding, neuroendocrine morphology, peripheral palisading, or ulceration between the nonaggressive and aggressive BCCs. Furthermore, there was no significant difference between groups in chromogranin expression or synaptophysin expression. None of the cases showed CK20 expression.

Conclusions: Our data showed that presence of tumor necrosis correlates strongly with an aggressive clinical course in patients with BCC. None of the other morphologic parameters or neuroendocrine markers analyzed were able to discriminate between the two groups.

521 Atypical Intradermal Smooth Muscle Neoplasms: Clinicopathologic Analysis of 85 Cases

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Background: Atypical or mitotically active dermal smooth muscle neoplasms are commonly termed "cutaneous leiomyosarcomas". However, preexisting - mostly small - series of these rare lesions suggest a low risk of aggressive behaviour.

Design: 85 cases were retrieved from the authors' consult files and institutional files. H&E sections were examined, immunohistochemistry was performed, and clinical details were obtained from referring physicians.

Results: There was a striking male to female preponderance (4.3:1), with a mean age of 56 yr (range 6-82, with 91% ≥40). 9 patients showed a prior history of malignancies (6 of the skin). Tumors measured 1.3 cm in average (11 tumors ≥2.0 cm) and were predominantly located on the trunk (32) and lower extremities (31). The remainder occurred on the upper extremities (17) or head & neck (4). Histologically, all tumors were either confined to the dermis or showed only very superficial subcutaneous extension. The majority showed an infiltrative growth pattern with fascicles of atypical spindle cells ramifying between collagen fibers. Necrosis was present in 10%. All cases showed cytologic atypia. Primary tumors showed a mean mitotic rate of 6/10 HPF. Recurrent tumors showed 14/10 HPF (and a greater degree of cytologic atypia). All tumors were immunopositive for SMA; 98% expressed desmin, 90% caldesmon, 27% pan-keratin (focal or scattered), and 1 focally S-100. Follow-up ranged from 5-156 months (mean 47). No metastases or tumor-related deaths were observed. Local recurrences were observed in 18 cases. All recurrences had developed prior to consultation, after a mean interval of 43 months. No recurrences were observed after consultation (mean follow-up of 32 months). 13 of the recurrent lesions showed positive margins in the primary excision and 3 showed margins <0.2 cm. Margin status was not available for 2 cases. The primary excisions of tumors which later recurred showed no increase in cellular atypia, presence of necrosis, or mitotic rate when compared to those which did not recur, nor were there any discernible clinical differences.

Conclusions: These tumors show a strong male predilection and predominantly occur on the trunk and lower extremities. When confined to the dermis or showing only minimal subcutaneous involvement, they appear to carry no risk of metastasis, hence the designation "sarcoma" is inappropriate. Margin status is the most important predictor of recurrence. Upon excision with clear margins, the risk of local recurrence is absent or very low. Hence we propose the term "atypical intradermal smooth muscle neoplasm".

522 SOX2 Expression in Melanoma Is Related to Tumorigenic Growth

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Background: *SOX2* is a gene located on chromosome 3q26.33 that encodes for a transcription factor important for embryonic neural crest stem cell pluripotency. Laser capture microdissection and genomic screening of stem cell-rich microdomains within the basal layer of squamous epithelium disclosed significant expression of SOX2. Immunohistochemistry confirmed SOX2 expression within rare basal cells that co-expressed microphtalmia transcription factor (MITF), but not mature melanocytic markers. This stimulated the hypothesis that SOX2 expression may be linked to melanocytic tumors.

Design: Human melanocytic nevi (n=40), vertical growth phase melanomas (n=151), and experimental xenografts derived from A2058 and SK-MEL-5 melanoma cell lines, expressing high and low levels of endogenous SOX2, respectively, were evaluated by immunohistochemistry. Stable SOX2-knockdown (KD) clones were generated in A2058 melanoma cells using a lentiviral shRNA approach. SOX2-KD efficiency was assessed by Western Blotting and mRNA levels of SOX2 and TIE1, a marker of melanoma cell plasticity and angiogenesis, were quantified using real time RT-PCR. Xenograft tumor volume was determined using the following formula: (maximal dimension x minimal dimension)^2/2. Statistical analysis was performed using ANOVA following log-transformation of data.

Results: Nuclear positivity for SOX2 was detected in 42% of melanomas compared to 15% of nevi, and SOX2 expression correlated with Breslow thickness. A2058-derived xenograft tumors achieved significantly greater mean estimated volumes than those derived from SKMEL5 cells ($p<.05$). Monolayer culture showed similar growth kinetics for SOX2 KD and control cells, but *in vivo* tumorigenicity assays revealed significantly diminished tumor growth in SOX2-KD tumors compared to controls ($p<.05$). SOX2-KD-derived tumors showed fewer TIE1-positive, CD31-negative patterned networks as compared to controls, a finding confimed by real time RT-PCR.

Conclusions: Human melanoma cells express the embryonic neural crest transcription factor, SOX2. SOX2 gene and protein expression correlate with melanoma growth in a xenograft model relevant to human disease. This effect may relate in part to a decrease in TIE1 gene and protein expression, suggesting a potential relationship between pluripotency and plasticity by melanoma cell subpopulations.

523 Multiple Skin Cancers Arising in Bone Marrow Transplant Recipients Can Exhibit a Donor Origin

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Background: Multiple skin cancers (basal cell carcinomas, squamous cell carcinomas and melanomas) and their precursor lesions often arise in bone marrow transplant recipients months to years after the transplant for reasons that are not understood. Approximately half of these patients receive a transplant from a sex-mismatched donor. We exploited this fact to gain insights into the nature of the skin carcinogenesis process in these transplant recipients.

Design: We studied 100 sex-mismatched transplant recipients who developed multiple (at least 5) skin cancers or precursor lesions over a period of months to years following successful transplant for diseases like relapsed lymphoma or leukemia. We created a tissue microarray (TMA) consisting of the secondary skin cancers or precursor lesions, the adjacent normal tissues and control skin cancers arising in non-transplant patients. We initially conducted X and Y chromosome FISH on this TMA. In selected cases we supplemented our FISH studies with studies that utilized informative but stable polymorphic microsatellite loci which could reliably distinguish donor from recipient; in other studies, we investigated possible donor lymphocytic or macrophage fusion with recipient stem cells by conducting gene rearrangement and ploidy studies in the skin cancers.

Results: Approximately 20% of the secondary skin cancers that developed were of donor origin. This was seen in both female as well as male recipients. The numbers may actually have been higher than observed because some skin cancers of male origin spontaneously lost the Y chromosome. This observation, not withstanding, cancers with the Y chromosome arising in females and cancers with XX signals arising in males supported their donor origin. Microsatellite marker studies further confirmed their donor origin. In these cases, there was no significant ploidy or immunoglobulin / T cell receptor monoclonal gene rearrangements, which excluded cell fusion or donor-recipient chimerism as mechanisms to explain the findings. Evidence of donor origin was seen in all of the histological types of skin cancers studied including their precursor lesions.
Conclusions: Our studies suggest that skin carcinogenesis arising in the transplant setting can take origin from pluripotent stem cells of bone marrow donor origin. These pluripotent stem cells first migrate into the skin where they subsequently transform.

524 Investigation of Histologic Correlates of Human Papillomavirus Infection in Penile Lesions Equivocal for Condyloma

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Background: Human papillomavirus (HPV) is considered the most common sexually transmitted disease in the developed world. Histologic distinction between condyloma acuminatum (CA) and seborrheic keratosis (SK) on penile skin is challenging when definitive viral-induced cytopathic changes are not present. Difficulty in classifying penile lesions equivocal for CA relates to lack of clear histologic criteria distinguishing warts from SK.
Design: SNOMED search through CoPath was used to identify penile specimens evaluated at FAHC between 1989-2006 with the following diagnoses: verruca vulgaris, CA, SK, or squamous papilloma. Each case was reviewed by three pathologists who assessed the following histologic features: architecture, character of stratum corneum and granular layer, and presence of immature parakeratosis (PK), coarse granules, koilocytes, dyskeratosis, melanin, horn pseudocysts, and inflammation. PCR was used to detect HPV in formalin-fixed, paraffin-embedded tissue blocks. PCR results were used to correlate viral presence with assessed histologic parameters.
Results: Out of 77 cases, there were 55 (71%) low risk HPV (LRHPV), 19 (25%) high risk HPV (HRHPV), and 3 (4%) negative for HPV by PCR. Histologic features most often seen in LRHPV lesions included rounded papillomatosis (62%), compact orthokeratosis (OK) with focal PK (44%), mild inflammation (64%), normal granular layer thickness (44%) with no evidence of coarse granules (64%), immature PK (73%), koilocytes (73%), increase in melanin (55%), and horn pseudocysts (58%). HRHPV lesions demonstrated generally similar histology but tended to show a slight preference for flat acanthosis (47%), OK (47%), and increased melanin along the basal layer (37%). Our predictions of viral presence based on histology revealed no false positives but did show 64% and 68% of false negative cases for LR and HRHPV, respectively.
Conclusions: The presence of koilocytes is a feature pathologists rely heavily upon in making a diagnosis of CA. The majority of our cases, morphologically equivocal for CA, were found to possess either LR or HRHPV by PCR. The standard morphologic criteria (given the present PCR data) are not predictive of HPV associated changes. It still remains a question whether HPV is a driver or mere passenger in the appearance of these penile lesions and expansion of this study using in situ hybridization and immunohistochemical stains to correlate with PCR data is ongoing.

525 Expression of P16 and MITF-1 in Acral Lentiginous/Mucosal Melanomas

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Background: Late research on the pathogenesis of melanoma suggests that acral lentiginous /mucosal melanomas (ALM/MuM) evolve through different mechanisms than the more frequent cutaneous melanomas. Loss of expression of p16 has been observed in melanomas occurring in intermittently sun exposed areas and may lead to cell-cycle progression. Microphthalmia transcription factor 1 (MITF-1) is a protein associated with the regulation of expression of p16. It is also considered a very sensitive marker of melanocytic differentiation. However, due in part to the scarcity of the occurrence of ALM/MuM in the United States, there is limited information on the expression of both p16 and MITF-1 in ALM/MuM.
Design: Immunohistochemistry for p16 and MITF-1 was performed on 34 sections of paraffin blocks corresponding to 22 patients with primary acral/ mucosal lentiginous melanomas showing invasive and/or in situ components, and in 9 sections of paraffin blocks corresponding to 9 patients with metastatic acral /mucosal lentiginous melanoma. Nuclear expression of p16 and MITF-1 was evaluated in the in situ and invasive components. Intensity and percentage of expression was scored.
Results: Patients with primary melanoma were 65 years old on average (35 – 87), 46.4% were male and 53.6% were female. Patients with metastatic melanoma were 66.9 years old on average (35 – 82), 55.6% were male and 44.4% were female. p16 was expressed in 91.3% (n=21) of in situ ALM/MuM, in 86.9% (n=20) of invasive ALM/MuM, and 77.8% (n=7) of metastatic ALM/MuM. MITF-1 was equally expressed in metastatic, invasive and in situ tumors.
Conclusions: There was a tendency to a loss of expression of p16 with progression of ALM/MuM. This loss of expression is similar to what has been described in the most common types of melanomas. MITF-1 was found to be a useful marker of melanocytic differentiation in ALM/MuM. In summary, in spite of the different molecular mechanisms implicated in the pathogenesis of ALM/MuM, immunohistochemical expression of p16 and MITF-1 shows similar characteristics to cutaneous melanomas of intermittently sun exposed skin.

526 Immunoreactivity of Smoothelin in Skin and Superficial Soft Tissue Tumors and Reactive Proliferations

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Background: The role of this study was to investigate the expression pattern of Smoothelin, a marker protein for contractile smooth muscle cells, in skin and soft tissue spindle cell proliferations, compare it to expression in the normal structures of skin and evaluate its possible role in the separation of true smooth muscle neoplasms from their mimics.
Design: A total of 43 cases with either myofibroblastic, fibroblastic, true smooth muscle, or glomus differentiation were examined by H&E and classified as follows: Dermatofibroma (5), Dermatofibrosarcoma protuberans (3), cutaneous leiomyoma (9), pilar leiomyosarcoma (3), glomus tumor (4), nodular fasciitis (3), hypertrophic scar (4), fibromatosis (5), oral fibroma (4), malignant fibrous histiocytoma (3). In addition, 6 cases of surgically removed unremarkable skin were used as normal controls. The scoring of Smoothelin reactivity was as follows: 3+ (strong cytoplasmic reactivity in >10% of cells), 2+ (moderate reactivity in >10% of cells), 1+ (weak reactivity in >10% of cells).
Results: Smoothelin reactivity was only expressed in true smooth muscle tumors and normal structures composed of true smooth muscle. It was consistently present in a 3+ pattern in the erector pili muscles and media of arteries in normal skin. All leiomyomas were Smoothelin positive (1+ to 3+ patterns). Two of three leiomyosarcomas were positive (1+ to 2+ patterns). All other cases were negative for Smoothelin.
Conclusions: Our results demonstrate that Smoothelin expression in the skin and soft tissue is limited to structures and neoplasms composed of true smooth muscle. This is analogous to the pattern of reactivity seen in other anatomic sites such as the bladder and gastrointestinal tract. It therefore can be used to distinguish true smooth muscle neoplasms from their mimics.

527 Merkel Cell Carcinoma (MCC) with Divergent Differentiation

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Background: MCC is a rare, aggressive tumor arising in sun-damaged skin of white elderly individuals. We report 14 cases of MCC with divergent differentiation, an unusual but well-documented phenomenon, to further characterise its clinico-pathological spectrum and relationship with polyomavirus (MCCPyV).
Design: 14 MCC with aberrant differentiation were retrieved from the consultation files of 4 of the authors. Clinical information was obtained from patient's notes or referring pathologists. PCR analysis for MCCPyV was performed from paraffin embedded tissue when available.
Results: 8 male and 6 females, all caucasian and with a mean age of 82.7 were included. MCC were located on the head and neck (8/14), extremities (3/14) and trunk (3/14). Follow up information was limited and only available in 11 cases. 8 patients underwent simple excision, 2 had surgery and adjuvant radiotherapy and 1 was only given palliative care. 7 patients (7/12) died of disease related causes within 1 year of diagnosis (4 of them with nodal metastasis, 1 with nodal and pulmonary metastasis and 1 with extension to the orbit) 2 died of unrelated causes after 4 and 5 years and 2 remained free of disease with follow up of 1 and 2 years respectively. All tumors showed the typical histological and immunohistochemical features of MCC with at least 1 divergent component. We found a single aberrant component in 8 cases (squamous in 7 cases and follicular in 1 case), 2 aberrant components in 5 tumors (glandular + squamous areas in 2, squamous + sarcomatous in 2, sarcomatous + rosettes in 1) and 1 case had 3 aberrant components (glandular + squamous + sarcomatous). All cases had dysplastic changes in the overlying epithelium and 8/14 showed epidermotropism PCR analysis for MCPyV was negative in all 12 cases tested, with positive controls.
Conclusions: 1) Clinical features are indistinguishable from classical MCC. 2) The most common aberrant differentiation is squamous 3) Coexistent SCC in situ and epidermotropism are more common than in classic MCC. 4) Our results suggest that MCPyV is absent in MCC with divergent differentiation. Similar findings have been reported by other groups. This fact questions the etiological role of MCPyV in this subset of MCC.

528 MicroRNA Profiles in Merkel Cell Carcinoma: Analysis Via Microarray and Novel Cloning and Sequencing Method

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Background: MicroRNAs (miRNAs) are small, non-coding, regulatory RNAs encoded in the genome of animals, plants and insects. Dysregulation of miRNA expression has been observed in many diseases, including cancer. We speculated that miRNAs are dysregulated in Merkel Cell Carcinoma (MCC), and that specific miRNAs are involved in MCC pathogenesis.
Design: To address this, we began by comparing the miRNA expression profiles of MCC tumors and normal skin by using archival formalin fixed, paraffin embedded (FFPE) tissue. We have previously confirmed the validity of using FFPE tissue to profile miRNAs using an Agilent array platform (J Mol Diagn. 2008 Nov;10(6):513-9). Quantitative real time RT-PCR analysis was used to validate the microarray data. Finally, the FFPE tissue samples underwent a novel cloning and sequencing profiling method to further confirm our results.
Results: Unsupervised hierarchical clustering clearly illustrated significant differences in miRNA expression profiles between MCC and normal skin. Supervised analysis identified 5 up-regulated and 10 down-regulated miRNAs in MCC relative to normal controls. The novel cloning and sequencing profiling technique confirmed both aberrantly overexpressed and underexpressed miRNAs in MCC. Certain miRNAs appear

to be of great interest in MCC, with levels of expression showing an increase in excess of 300 fold, while others were up to 500 fold decreased, as compared to normal skin.
Conclusions: Our studies suggest that miRNAs are dysregulated in Merkel Cell Carcinoma and may play a role in its initiation and progression.

529 Touch Preparation Analysis of Skin Lesions Is a Rapid, Accurate Diagnostic Technique: A Prospective Clinical-Pathologic Study

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Background: The cytologic analysis of skin based lesions has been largely confined to microbial analysis such as KOH preparation, or Tzanck smears. Skin based cytology methods for the diagnosis of epidermal/dermal lesions are under-developed.
Design: We sought to evaluate the use of touch preparations of skin biopsies as a method for accurately diagnosing lesions. Touch preparations were performed on 56 skin biopsies which included squamous cell carcinomas, basal cell carcinomas, nevi, drug eruptions, and ulcers (among others). Specimens were processed using Diff-Quik solutions. Three independent pathologists, blinded to the final pathologic diagnosis, reviewed slides and were instructed to render a diagnosis using the following categories: 1) Negative for malignancy 2) Reactive 3) Atypical 4) Suspicious for malignancy 5) Diagnostic of malignancy. In addition, pathologists commented on the cellularity of lesions and the general morphologic findings in a separate paragraph. The gold standard for the diagnosis of lesions was considered to be the findings on permanent histology.
Results: The overall specificity for the diagnosis of squamous cell and basal cell carcinomas was 97%; the sensitivity of these diagnoses was 100%. Lesions categorized as reactive corresponded to ulcers without carcinoma, recent biopsy sites, and irritated seborrheic keratoses. In addition, one lesion identified as atypical/suspicious for carcinoma by cytology was histologically bland on initial sections, but after deeper sectioning was shown to be atypical, thus demonstrating the utility of this touch preparation technique. Finally, basal cell carcinomas have a clearly distinct cytomorphology which allows for the confident diagnosis of these lesions; in this study, this technique was 100% sensitive and 100% specific for the diagnosis of basal cell carcinomas.
Conclusions: Touch preparation cytology of skin biopsies is a rapid, accurate, cost-effective methodology for initial screening diagnosis of patients with skin disorders. Such studies allow for the appropriate triage of specimens which may require additional studies. Finally, we describe the cytomorphology of basal cell carcinomas in depth, allowing for the accurate and consistent diagnosis of these lesions. Expansion of this series of cases will help further demonstrate the novel utility of this technique.

530 Value of P63 and Podoplanin (D2-40) Immunoreactivity in the Distinction between Primary Cutaneous Tumors and Carcinomas Metastatic to the Skin: A Clinicopathologic and Immunohistochemical Study of 79 Cases

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Background: The distinction of metastatic carcinomas to the skin from poorly differentiated primary cutaneous carcinomas and sometimes primary benign adnexal tumors can pose a significant diagnostic challenge. The purpose of this study was to evaluate the role of P63 and podoplanin (D2-40) immunoreactivity for separating primary skin tumors versus cutaneous metastases of carcinomas from internal organs.
Design: Thirty seven primary tumors and 42 cutaneous metastatic carcinomas were evaluated. The 37 primary cutaneous tumors included 14 cases of benign adnexal tumors, 9 malignant skin adnexal neoplasms, and 14 primary squamous and basal cell carcinomas. The 42 metastatic carcinomas all corresponded to metastases from internal organs in patients with a well-documented history of a primary tumor at another location.
Results: We found variable positivity with podoplanin in all primary cutaneous neoplasms including spiradenoma (6/6), hidradenoma (2/4), cylindroma (3/3), desmoplastic trichilemmoma (1/1), poorly-differentiated squamous cell carcinoma (4/4), sebaceous carcinoma (1/1), basal cell carcinoma (4/10), trichilemmal carcinoma (2/2), eccrine carcinoma (3/3), microcystic adnexal carcinoma (1/1), adnexal carcinoma NOS (1/1), and porocarcinoma (1/1). In contrast, all visceral metastatic carcinomas were negative (0/42) for podoplanin. In regards to P63, all cases of primary cutaneous tumors were positive for P63 (37/37); in contrast, all cutaneous metastatic visceral carcinomas were negative (0/42). Sensitivity, specificity, and positive and negative predictive value of podoplanin and P63 immunoreactivity to separate primary skin neoplasms from metastatic carcinomas was 78.4%, 100.0%, 100.0%, and 84.0% for podoplanin, respectively, and 100.0%, 100.0%, 100.0%, and 100.0% for p63, respectively.
Conclusions: The differences in p63 and podoplanin immunohistochemical expression between primary skin tumors and metastatic carcinomas to the skin were statistically significant ($p<0,0001$). The results of our study suggest that the combined expression of P63 and podoplanin are a useful adjunct for the diagnosis of skin tumors in the clinical setting of a questionable metastasis and may be relatively specific for distinguishing primary skin tumors from metastatic carcinomas to the skin.

531 Immunolabeling Pattern of Podoplanin (D2-40) May Distinguish Basal Cell Carcinomas from Trichoepitheliomas: A Clinicopathologic and Immunohistochemical Study of 49 Cases

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Background: Basal cell carcinomas (BCC) and trichoepitheliomas, most of the time are effectively distinguished based on defined histologic criteria; however, they may pose diagnostic challenge in rare occasions when the morphologic distinction between these two neoplasms are not clear. Their distinction is clinically important because the risk of progressive disease in BCC can be problematic, and trichoepitheliomas misinterpreted as BCC burdens the patient with an inaccurate diagnosis that may result in inappropriate surgery. Podoplanin (D2-40) is a well known lymphatic endothelial surface marker that has also been postulated to be upregulated in the outer root sheath of hair follicles and cutaneous neoplasms, such as adnexal tumors, squamous cell carcinomas, etc.
Design: We studied the expression of podoplanin (D2-40) by immunohistochemistry to determine if this marker can reliably separate these neoplasms. Immunolabeling for podoplanin was scored as negative, focally positive or diffusely positive (cytoplasmic membrane staining). We defined negative as no reactivity, focally positive as staining in 1% to 25% of the tumor cells and greater than 25% was defined as diffuse staining. A total of 49 cutaneous tumors, including 22 cases of trichoepitheliomas and 27 cases of follicular BCC were examined. Of the 27 cases of BCC, 18 cases were located in the head and neck area, 5 in upper extremities, and 4 in the back. Of the 22 cases of trichoepitheliomas, all the 22 cases were located in the head and neck area.
Results: Podoplanin expression was present in 21/22 cases or trichoepitheliomas; 11 cases were diffusely positive (50%), 10 cases were focally positive (45.45%), and 1 case was negative (4.54%). Podoplanin expression was present in 6/27 cases of BCC; 2 cases were diffusely positive (7.40%), 4 cases were focally positive (14.8%), and 21 cases were negative (77.7%).
Conclusions: In summary, podoplanin expression was only weakly and focally positive in BCC (22% of cases) and diffusely and weakly positive in trichoepitheliomas (95.45% of cases). The sensitivity and specificity of podoplanin immunoreactivity to separate trichoepitheliomas from BCCs was 95.5% and 77.8% respectively. This data suggests that podoplanin expression could be a useful and potential marker to distinguish BCC from trichoepitheliomas, especially when there is a high index of histologic suspicion for either of these tumors.

532 "Neuroendocrine" Basal Cell Carcinoma: A Novel Morphologic Variant?

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Background: Basal cell carcinoma (BCC) is the most prevalent cutaneous neoplasm in the US. BCCs are usually composed of basaloid cells with hyperchromatic nuclei and relatively little cytoplasm. We have encountered few cases characterized by high nuclear to cytoplasmic ratio, nuclear molding and a homogeneous chromatin pattern with inconspicuous nucleoli reminiscent of neuroendocrine carcinoma. Immunohistochemical stains were negative for CK 20 ruling out Merkel cell carcinoma; however, the tumors expressed chromogranin. This observation prompted the current study which aims to evaluate the presence of "neuroendocrine" features in BCCs.
Design: A total of 53 consecutive cases of aggressive and nodular BCC histologic variants were selected for the study. All cases were evaluated for histologic pattern, mitotic index/ mm2, presence of apoptosis, necrosis, squamous differentiation, ulceration and peripheral palisading. The presence of neuroendocrine (NE) morphology (high N/C ratio, nuclear molding, homogeneous chromatin pattern, inconspicuous nucleoli) was assessed by 3 separate investigators and a consensus was reached. In addition immunohistochemical stains for chromogranin, synaptophysin and CK20 were performed in all cases.
Results: NE morphology was noted at least focally in 50% of cases. CK 20 and synaptophysin were positive in 0% and 4% of cases, respectively. The differences in cases with NE versus without NE morphology are shown in the table.

	Mitoses /mm2	Prominent apoptosis	Squamous differentiation	Peripheral palisading	Aggressive histology	Chromogranin expression
All cases	8.5	18%	46%	51%	55%	52%
Positive NE	13	29%	36%	39%	64%	79%
Negative NE	4	7%	54%	61%	46%	25%
p-value	0.0001	0.03	0.1	0.1	0.1	0.001

In a multivariate analysis, high mitotic index and chromogranin positivity were independent predictors of NE morphology.
Conclusions: A neuroendocrine morphology in BCC is associated with expression of chromogranin, increased mitotic rate and prominent apoptosis. This group of BCCs appears to have an "incomplete" neuroendocrine phenotype as they are all negative for CK20 and only seldom express synaptophysin. Our study also is the first to document a relatively high frequency of chromogranin positivity in BCC tumors (52%). Further studies to assess the biological significance of NE phenotype are warranted.

533 Prevalence and Genotype Identification of HPV Infection in Penile vs. Vulvar Carcinomas

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Background: Human papillomavirus (HPV) has been detected in cases of penile and vulvar carcinomas, and it appears that certain types of HPV are an important risk factor for the development of these tumors. The aim of this study was to compare the prevalence of HPV and the implicated genotypes in penile and vulvar carcinomas. In order to make that comparison possible, we studied patients from the same regional area and we used the same methodology in all cases. This study will provide valuable information to determine the possible correlation that carcinomas of male and female external genitalia may present.
Design: Samples from 49 patients with penile carcinoma and from 37 patients with vulvar carcinoma were selected. Formalin-fixed, and paraffin-embedded specimens were collected from the archives of the Pathology Department of our Hospital. DNA was

extracted from the paraffin blocks, and PCR technique was performed in conditions that prevented contamination. All the cases of this study were tested with two different sets of consensus primers (GP5+/GP6+ and My09/My11) in order to improve the sensitivity of the technique. Appropriate positive and negative controls were run in parallel. To determine the implicated genotypes positive cases were sequenced.

Results: The overall prevalence of HPV was found to be 77.5% in penile carcinoma and 30.3% in vulvar carcinoma. From the HPV positive patients with penile carcinoma, genotype 16 was detected in thirty-two cases (84,2%), genotype 18 in four (10,5%), and in two subjects, results were inconclusive (5.2%). Besides HPV16 or 18, there were no other genotypes detected in any of the cases of penile carcinoma; however, HPV6 and HPV11 were found in benign penile lesions that were run in parallel. Genotype characterization of vulvar carcinoma cases showed that HPV16 was present in two patients (6,1%), HPV18 in four patients (12,1%), HPV33 in three patients (9,1%), and HPV 35 in one patient (3,0%).

Conclusions: This study shows that the HPV prevalence detected in vulvar carcinomas is lower than that detected in penile carcinomas, and that the HPV genotypes identified in these tumors are markedly different. The results show low HPV correlation between carcinomas of male and female external genitalia, and indicate that HPV participate differently in the pathogenesis of these tumors.

534 p63 Expression in Merkel Cell Carcinoma Is Related to Prognosis: An Immunohistochemical and Molecular Analysis

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Background: p63 expression in Merkel cell carcinoma (MCC) indicates an aggressive behavior of the tumor. At least three TA variants (TAp63α,β,γ) and three ΔN variants (ΔNp63α,β,γ) by alternative splicing from p63 gene have been identified. In addition recently it has been suggested that presence of polyomavirus (MCPyV) in MCC tumor tissue is an indicator of adverse prognosis. Therefore, to better define the role of p63 and its variants in MCC and the possible relation to MCPyV, we examined a series of MCC from one single institution.

Design: 18 cases of MCC from 15 patients (2 cases showed nodal metastases and 1 case brain metastasis) were investigated for p63 expression by immunohistochemistry (IHC) and by reverse-transcription polymerase chain reaction (RT-PCR) using isoform-specific primers to evaluate the p63 mRNA expression patterns. Probes for p63 gene (3q28) were used for FISH analysis to evaluate the p63 gene status. The presence of MCPyV in the MCC tumor genome was also investigated by PCR in all cases.

Results: p63 expression was detected in 11/18 (61%) cases using IHC and p63 positivity was associated with decreasing overall survival (p=0.003). All these 11 cases presented at least one of the p63 isoforms, both in the primary MCC (8 cases) and in metastases (3 cases), with a variable expression pattern of the isoforms: TAp63α was detected in 7/11 cases, ΔNp63β in 3/11 cases and ΔNp63α in 1/11 case. No p63 gene amplification was found by FISH analysis. All these patients died of disease after average follow-up of 31 months (range: 2-142 months). The remaining 7 cases of MCC, showing negativity for p63 at IHC, did not display any p63 isoform in 5 cases while 2 cases showed only ΔNp63 isoforms with different C-terminals (α and γ, respectively). Four of the patients are alive without disease, 2 died of other causes and one is alive with nodal metastasis. The average follow-up was 42 months (range: 8-67 months). Clonal integration of MCPyV DNA sequences was observed in all cases.

Conclusions: The present IHC and molecular data confirm p63 expression in a group of MCC with aggressive clinical behavior and suggest that a transcriptional dysregulation of p63 gene is involved in the pathogenesis of MCC. IHC analysis is less sensitive than the molecular analysis to value p63 expression in MCC cases. Clonal integration of MCPyV DNA sequences does not seem related to prognosis.

535 Interdigitating Dendritic Cell Sarcoma of the Skin: A Rare and Challenging Malignant Tumour That Demonstrates a Distinctive Dendritic Pattern of Staining with WT-1 and CD99

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Background: Interdigitating dendritic cell sarcoma (IDCS) is a rare malignant tumour representing less than one percent of hematogenous malignancies. Its occurrence in extranodal sites is extraordinarily rare and these generally represent metastatic foci. Only three cases of primary involvement of the skin are present in the literature. Diagnostic recognition of this entity is problematic due to subtle morphological and immunophenotypic features which may easily be overlooked or mistaken for more common entities.

Design: Histological, immunohistochemical and ultrastructural studies were performed on three routinely processed excisional biopsies. A clinical review was also performed.

Results: Histomorphology and immunophenotype were consistent with the diagnosis of Interdigitating dendritic cell sarcoma in all cases. Ultrastructural features were also compatible. S-100, CD68, CD99, and WT-1 immunohistochemical staining were diffuse and strong in a staining pattern that highlighted the interdigitating cellular processes. Two cases were found to represent primary cutaneous disease with no evidence of underlying nodal or visceral disease. The third case was found to represent nodal disease originating from an intraparotid lymph node.

Conclusions: Here, we report only the fourth and fifth cases of a primary cutaneous IDCS as well as an additional case presenting within an intraparotid lymph node that was initially diagnosed as melanoma. IDCS represents an under-recognized and challenging malignant tumour that can easily be misdiagnosed due to overlapping features with more common cutaneous entities. We report for the first time immunohistochemical positivity of this tumour for WT-1 and CD99. Furthermore, WT-1 in particular, demonstrates a distinctive dendritic pattern of staining that can be used as a useful discriminating feature from other cutaneous lesions in the differential diagnosis.

536 Comparative Analysis of Spitz Tumors: A Clinicopathologic Follow-Up of 163 Cases

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Background: The discrimination of the benign from malignant variants of Spitz tumor has been a great challenge in dermatopathology. A few grading systems have been proposed and categorized them into classic Spitz tumor/nevus without atypicality (CST) with low, atypical Spitz tumor (AST) with intermediate, or Spitzoid melanoma with high risk, based on a combination of clinical and pathologic parameters, however, there is lack of consensus for gauging their malignant potential. Studies on Spitz tumors have suffered from short follow-up period and/or limited number of cases. There is a chance of lymph node metastasis in a subset of cases, but its impact on survival is unknown. The current study is designed with the goal to compare the classic Spitz tumor/nevus (CST), and the atypical Spitz tumor (AST) with clinicopathologic follow-up in regards to their behavior and prognosis.

Design: During1987-2002, all cases of primary Spitz tumor in the database of Pathology Dept at MGH were included. CSTs and ASTs were diagnosed and discriminated based on organizational, proliferation, and cytologic criteria as previously published. The database of the Pigmented Lesion Clinic at MGH was cross-linked to obtain clinical follow-up information on each patient.

Results: Spitz tumors (n=163, median age=29.1 yrs) with long term clinical follow-up (median= 6.7 yrs) were included and were divided in CST (n=71) and AST (n=92) groups. CSTs mean age was 24.2 yrs vs. 32.9 for AST (p<.001). Lesions were common on the extremities (94/163, 57.7%) with no difference in site predilection (p=NS). Sentinel lymph node biopsies were performed on 6/92 patients with AST; one person had a nodal involvement who underwent a negative completion lymph node dissection and one year of high dose interferon, currently 8 yrs post diagnosis and disease free. One case of metastases in a patient with AST and concurrent history of an intermediate thickness melanoma (1.2 mm, level IV) was seen. All other patients were disease free.

Conclusions: We report the largest series with the longest follow up data to date on the outcome of Spitz tumors. After a median follow-up period of 6.7 yrs, we did not detect any fatalities; only one patient in stage IV disease who had a concurrent intermediate thickness melanoma. Prognosis is thus, highly favorable for both the classic and atypical Spitz tumors.

537 Differential Gene Expression Profiles of Neurothekeomas, Nerve Sheath Myxomas, Cellular Fibrous Histiocytomas and Schwannomas by Microarray Analysis

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Background: Traditionally, neurothekeomas (NTK) and nerve sheath myxomas (NSM) were considered related cutaneous neoplasms of peripheral nerve sheath (PNS) origin based on light microscopic resemblances. Immunohistochemical and ultrastructural data indicate NSMs truly exhibit nerve sheath differentiation, while no such compelling evidence exists for NTKs. Although NTKs lack a specific immunohistochemical profile, similar antigen expression and histopathological patterns suggest NTKs may be categorized as fibrohistiocytic tumors (FHT). To date, no known studies have examined the histogenetic relationship of these tumors by utilizing molecular based techniques such as microarray-based gene expression profiles on formalin-fixed paraffin-embedded (FFPE) tissues.

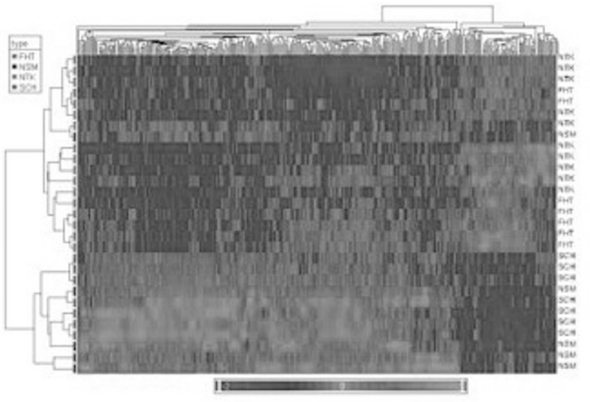
Design: Cases of schwannomas (SCH), NSMs, mixed/cellular NTKs, and cellular FHTs diagnosed in the past 3 years were identified in our database. Archival FFPE tissue from 29 patients were selected for microarray analysis (7 SCH, 5 NSMs, 10 mixed/cellular NTKs, and 7 cellular FHTs). Following tumor RNA isolation, amplification, and labeling using commercially available kits, labeled targets were hybridized to the Affymetrix GeneChip® Human Genome U133 Plus 2.0 Array. Acquisition of array images and data analyses were performed using appropriate software.

Results: Hierarchical clustering indicated discrete groups which correlated with histopathologically identified diagnoses. NSMs demonstrate very similar molecular genetic signatures to SCH, while NTKs, although distinct, more closely resemble FHTs.

Table 1

Histopathological Diagnoses	# Genes Upregulated (>3FC, p<0.01)	# Genes Downregulated (>3FC, p<0.01)
NTK vs NSM	73	289
NTK vs FHT	22	297
NTK vs SCH	258	578
NSM vs SCH	0	96
NSM vs FHT	179	336

*FC=fold change



Conclusions: We are the first to report distinct gene expression profiles for NSMs and NTKs, which further substantiates the argument that these are separate entities. We offer molecular evidence suggesting NTKs may actually be a variant of FHTs rather than of PNS origin, unlike NSMs. Additional studies of NTKs and FHTs are planned to help determine if NTK is a distinct entity.

538 Wilm's Tumor 1 (WT1) Expression in Endocrine Mucin-Producing Sweat Gland Carcinoma (EMPSGC) – Immunohistochemical Evidence of Tumor Progression

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Background: EMPSGC is a rare, under recognized, low-grade sweat gland carcinoma that occurs predominantly in eyelids. Areas of benign eccrine cysts (hidrocystoma), atypical intraductal proliferation and mucinous carcinoma can be seen associated with EMPSGC suggesting tumor progression, similar to endocrine DCIS of the breast, which often coexists with mucinous carcinoma. WT1 protein, a transcription factor, is overexpressed in many solid tumors and it seems to play a role in oncogenesis. Its expression in skin adnexal tumors has not been studied.

Design: Computer based search performed for adnexal eyelid tumors, excluding basal cell carcinoma, 1989 to 2009, identified 11 EMPSGC. Demographic and clinical findings were obtained from pathology reports and patients electronic records. Biopsies were evaluated for several histological features. Immunohistochemistry for WT1, chromogranin, synaptophysin, ER, EMA, PCEA, CK7, CK20, p53, and MIB-1 were performed.

Results: There were 6 women and 5 men; mean age 62 years (range, 40 to 82). Most cases presented as slow-growing eyelid mass, in the lower (6) or upper (2) eyelid, medial (1) or lateral canthus (1), and eyelid NOS (1). Original diagnosis included hidradenoma (8), atypical hidradenoma (1), and eccrine carcinoma (2). Histologically, EMPSGC were characterized by dermal nodules with solid, cystic, and papillary areas. Tumor cells were uniform, polygonal shape with pale eosinophilic cytoplasm and round nuclei showing no or mild cytological atypia. Extra and intracellular mucin was observed; rare mitosis; no tumor necrosis. Areas of hidrocystoma (4), atypical intraductal proliferation (3) and mucinous carcinoma (3) were present. All tumors were positive for WT1, CK7, ER, p53 (weak), PCEA and EMA; and negative for CK20. Synaptophysin was positive in 9 cases and chromogranin in 8. Mib-1 proliferation index was low in 8 cases and moderate in 3. Of interest, strong WT1 nuclear staining was observed in all EMPSGC, including areas of mucinous carcinoma and areas of atypical intraductal proliferation. No WT1 staining was observed in areas of hidrocystoma, adjacent adnexal structures or overlying epidermis.

Conclusions: Our study shows WT1 expression in the neoplastic epithelial cells of EMPSGC, including areas of atypical intraductal proliferations and mucinous carcinoma. The absence of WT1 expression in areas of benign cyst/hidrocystoma and normal cutaneous structures, suggests that WT1 up-regulation plays a role in tumor cell proliferation and progression of EMPSGC.

539 The Histomorphologic Spectrum of Primary Cutaneous Diffuse Large B-Cell Lymphoma (DLBCL), "Leg Type" and "Other" Category: A Histopathologic and Immunohistochemical Study of 79 Cases

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Background: Primary cutaneous diffuse large B-cell lymphomas have been historically matter of debate in the literature. Recently, in 2005 the WHO-EORTC classified cutaneous B-cell lymphomas into the following main 3 groups: primary cutaneous marginal zone B-cell lymphoma, primary cutaneous follicle center cell lymphoma, and primary cutaneous diffuse large B-cell lymphoma, leg type. In the WHO-EORTC classification the term PCLBCL other is used in rare cases of PCLBCL that do not belong to the leg type group or the group of PCFCL. In this study we assessed retrospectively the morphologic, immunophenotypic, and the clinical features of 79 cases of PCDLBCLs including the leg type and the other category in order to better categorize the histologic and clinical spectrum of this unusual neoplasm.

Design: 79 cases were analyzed (M: F = 37 : 42, range age: 34 to 94). 53 cases were classified as leg type and 26 cases were classified as other by using the WHO-EORTC classification. Of the 53 cases classifies as leg type 33 were Females and 20 were Males; of the 26 cases of other NOS type, 9 were Females and 17 were Males.

Results: PCDLBCL leg type was characterized by an older age on onset (mean 64), female predominance, predilection for the leg (58.4%), high proportion of bcl-2 expression and extracutaneous dissemination. The PCDLBCL NOS type was characterized also by advanced age of onset (mean 58), male predominance, and predilection to head and neck area. Most of the cases showed the classic morphologic appearance of PCDLBCL, but cases mimicking Burkitt lymphoma (starry-sky pattern), subcutaneous t-cell lymphoma, NK lymphoma, mycosis fungoides (epidermotropism), low-grade B-cell lymphomas, epithelial malignancies, Merkel cell carcinoma, etc were encountered in this series. Immunohistochemical stains plus careful histologic examination helped to establish the correct diagnosis.

Conclusions: The histologic diagnosis of PCDLBCL poses little diagnostic difficulty; however, some cases may adopt unusual or unfamiliar appearances mimicking other lymphoproliferative disorders. We believe the differential diagnosis of PCDLBCL sometimes can be broad and difficult to define both clinically and histologically. To the best of our knowledge this is the largest series of PCDLBCL reported in the literature.

540 Histologic Review of the First Near Total Face Transplant

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Background: Recent advances in immunosuppression and surgical techniques have progressed to make a near total face transplant, like the one performed at the Cleveland Clinic in December of 2008, possible. Composite tissue grafts consist of skin, subcutaneous tissue, muscle, nerve, and bone. Accurate clinical and histologic rejection surveillance is vital to preserve the function of the graft. A histologic review of the first eight months following the first near total face transplant was conducted and is discussed below.

Design: Paired skin and mucosa biopsies were obtained with accompanying clinical photographs at weekly, then biweekly, then monthly intervals. Paraffin sections for H&E, PAS, immunoperoxidase, and unstained sections for the TUNEL assay were obtained. Biopsies were graded using the Banff 2007 working classification of skin-containing Composite Tissue Allograft (CTA) Pathology. Immunostaining for CD3, CD4, CD8, CD20, CD68, CD30, FoxP3, K167, HMB45, CD1a, S100, FactorXIIIA, CD31, and CD34 was performed. The TUNEL assay was also performed to assess apoptosis.

Results: No definitive evidence of rejection was seen clinically. Histologically, grade II rejection was identified on five different biopsy dates (days 20, 63, 77, 91, 209, 246) with grade III rejection on two occurrences (days 47 & 153-159) with apoptosis that was confirmed by TUNEL. Interestingly, significant perivascular inflammation was not identified, even in the cases of grade III rejection. However, the inflammation has been noted in multiple small to medium size foci of interface inflammation with apoptosis. Furthermore, the significant episode of histologic rejection was only present in the mucosal biopsies and was absent from the paired skin biopsy.

Conclusions: We reviewed the histology of the first near total face transplant and documented five episodes of grade II rejection and two episodes of grade III rejection. Histologic evidence of rejection was only identified in mucosal biopsies, and was absent from the paired skin biopsies. Despite the presence of interface inflammation with clumps of apoptotic cells, significant accompanying perivascular inflammation and clinical symptomatology were not identified. We believe that the Banff (CTA) 2007 classification system is the most accurate grading system for composite grafts involving the face at this time. As more face transplant procedures are preformed, the difference between histologic evidence of rejection in mucosal and skin biopsies may be further elucidated.

541 Identifying Merkel Cell Polyoma Virus by FISH and PCR

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Background: Merkel cell carcinoma (MCC) is a rare and deadly neoplasm of the skin that frequently affects the elderly and the immunosuppressed. Recently, studies have shown a relationship between MCC and a new polyoma virus named Merkel cell polyoma virus (MCPyV). Between 40 and 85% of MCCs contain the virus. We examined all of the primary resections of MCC in our archives for the presence of the virus.

Design: Twenty one primary cases of MCC were identified from 1980-present. Real time PCR was performed using a previously published primer set MCVPSI (109 bp amplicon). Selective PCR products were sequenced to confirm the presence of MCPyV. FISH was performed using a SpectrumGreen (Abbott Molecular) labeled probe that was developed in-house from plasmids available from the NIH AIDS Research & Reference Reagent Program. Cell blocks from a MCC cell line (MKL-1) positive for MCPyV served as the positive control. Cell blocks from a MCPyV negative cell line (MRC-5) were the negative control.

Results: The average age, at diagnosis, was 74 years. Ten (59%) of the seventeen cases with adequate clinical histories had immune dysfunction. Reasons for the immune dysfunction included: immune suppression (4), CLL (1), MDS (3), dialysis (1), and cirrhosis (1). Males and females were affected equally. FISH assay demonstrated positivity for MCPyV in 48% of cases. FISH also demonstrated that the virus was only present in the tumor cells and not in the surrounding dermal tissue. The PCR was more sensitive. PCR amplified MCPyV DNA in each of the cases identified by the FISH assay along with five additional cases for a total of 71%. Interestingly, 84% of the immunocompetent cases were positive for the virus versus 60% in the immunocompromised population. There was no significant difference in age between MCPyV positive and negative cases. The presence of MCPyV was not a significant prognostic factor for survival or metastasis.

Conclusions: We endeavored to utilize our case files to explore MCC and its association with MCPyV. We confirmed that MCC is a disease of the elderly and immunosuppressed. FISH was positive in 48% of cases and 71% of cases contained MCPyV by PCR. Paradoxicaly, immunocompetent individuals are more likely to have the MCPyV in our series. This seems counterintuitive and needs further study due to the small sample size. We were not able to deduce a significant prognostic significance for the presence

of the virus. The finding of 71% of MCCs being positive for MCPyV is consistent with the literature.

542 Stem Cell Marker ALDH1 Expression in Melanoma

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Background: The issue of the expression of stem cell markers in melanoma is controversial. The recent publication of the aberrant expression of CD 20 in a subpopulation of melanoma-initiating cells also adds to the controversy (J Clin Oncol. 2008;26:2890-2894). Understanding and characterizing the various possible subpopulations within malignant melanoma and the dynamics of clonal dominance is currently being investigated to gauge the early and advanced characteristics of stem cell markers at these different stages. Aldehyde dehydrogenase-1 (ALDH1) has recently emerged as a possible marker for breast cancer, brain tumors and for identifying colonic stem cells in colorectal carcinoma. However, to our knowledge, ALDH1 has not been previously studied as a stem cell marker in melanoma.

Design: 35 patients were selected from a Veteran Administration Medical Center Hospital in Detroit, Michigan, with disease ranging from melanoma in situ, to nodular melanoma and metastatic melanoma. CD 20 and ALDH1 are two immunostains applied to the microslides prepared from paraffin-embedded tissue from these 35 patients.

Results: None of the cases (0/35, 0%) stained for CD 20. 20/35 cases (57%) stained moderately-to-diffusely for ALDH1 (staining 10-40% of the tumor cells). This group encompasses the patients with nodular and metastatic melanoma. 5/35 slides (14%) had focal-to-patchy staining for ALDH1, this group is comprised of the patients with early lesions (from melanoma in situ, up to Stage 1). Of these early lesions cases, 2/4 (50%) had subsequent advanced disease, or metastatic melanoma. 10/35 cases (29%) did not stain for ALDH1.

Conclusions: Our cases of melanoma do not show aberrant CD 20 expression, disproving the assumption of CD 20 as a marker for cancer stem cells in melanoma. However, ALDH1 was expressed in advanced melanoma, which translates to a higher percentage of melanoma cells being stem cell like. Fewer of the early lesions stained with ALDH1, but from the patient follow up of this group, 50% developed advanced disease. Therefore, ALDH1 can potentially be used as a prognostic stem cell marker to predict metastatic melanoma in early lesions.

543 Evidence of Regulatory T-Cell Immunophenotype in a Subset of HTLV-I-Associated Infective Dermatitis: An Early Sign of Progression?

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Background: Infective dermatitis associated with HTLV-I (IDH) is a childhood eczema that rarely presents in adults. It has been postulated that IDH may represent a cofactor for the subsequent development of cutaneous adult T-cell leukemia/lymphoma (ATLL.) The status of CD25-positive regulatory T cells in this entity is unknown, despite the well-established fact that ATLL cells usually display a CD4, CD25-positive immunophenotype. Here, we report the histopathological and immunohistochemical findings of IDH in a cohort of affected children and adults from Peru.

Design: Sixteen skin biopsies from fifteen patients were examined. Patients ranged in age from 5 to 82 years. All the patients were positive for HLTLV-I by serology. Histological assessment and evaluation of CD3, CD4, CD8, and CD25 by immunohistochemistry were performed. CD25 expression was scored as focal (fewer than 10%), moderate (10 to 50%), and diffuse (greater than 50% of the lymphocytes.).

Results: The lymphocytic infiltrate was categorized as lichenoid (8/16 cases), superficial and deep perivascular (7/16), and mixed (1/16.) Neutrophils in stratum corneum, parakeratosis, and spongiosis were seen in 11/16 biopsies. Fibrosis of the papillary dermis was identified in 6/16 cases. Exocytosis of lymphocytes in the epidermis, at least focal, was present in all the cases. Folliculotropism was present in two cases. The lymphocytic infiltrate was predominantly composed of CD3-positive T cells. The epidermotropic cells were mainly CD8-positive T cells. Only very rare CD4-positive cells were present in most of the cases (10/16.) In six cases, a population of CD4-positive T cells was seen in the dermis showing focal epidermotropism. From these six cases, four (4/16, 25%) displayed a moderate to diffuse expression of CD25 by the infiltrating lymphocytes.

Conclusions: Our study demonstrates that a subset of IDH shows a population of CD4-positive, CD25-positive T cells with possible regulatory immunophenotype. This population may contribute to an impaired cell-mediated immune response and potentially to progression to ATLL. Expression of CD25 by a subset of IDH may have therapeutic implications in the management of these patients.

544 Stem Cell-Associated Markers Distinguish Melanoma from Malignant Peripheral Nerve Sheath Tumor

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Background: Melanoma can have varied histologic appearances and often causes diagnostic confusion. When melanoma metastasizes to deep soft tissue or internal organs, a major differential diagnosis is with malignant peripheral nerve sheath tumor (MPNST) as both tumors may have spindle cell morphology and express S100 protein. The differential diagnosis becomes more difficult if additional melanoma markers such as melan A and HMB45 are equivocal. The cancer stem cell model states that a minority of cancer cells are cancer stem cells (CSC) with tumor initiation potential. However, melanoma is unique in that single unselected melanoma cells can initiate tumors in in-vivo models with high frequency, suggesting that melanoma cells have CSC properties. A previous study showed that stem cell-associated markers can differentiate melanoma from nevi. The current study was performed to determine if melanoma can be differentiated from MPNST with such markers.

Design: Two TMAs were used for the study, one containing 78 cases of melanoma and the other 68 cases of MPNST. The TMAs were stained with antibodies for EZH2 (VisionBio, 1:100), CD44 (eBioscience, 1:1000), SOX2 (R&D Systems, 1:100), C-Kit (Dako, 1:100) and Oct3/4 (BioCare, prediluted). Only tissue cores being at least 50% intact were scored.

Results: The results are summarized in Table 1. Among the stem cell-associated markers tested, SOX2 was expressed in 80% (55/69) of melanomas and 18% (12/66) of MPNSTs ($p<0.001$). C-Kit was expressed in 65% (50/76) of melanomas but none of the MPNSTs ($p<0.001$). Expression of other 3 markers was not statistically different between the two entities.

Table 1. Expression of stem cell-associated markers in melanoma and MPNST

	SOX2	EZH2	C-Kit	CD44	OCT3/4
Melanoma	80% (55/69)	87% (68/78)	65% (50/76)	100% (77/77)	0% (0/78)
MPNST	18% (12/66)	56% (38/67)	0% (0/48)	94% (62/66)	0% (0/67)

Conclusions: 1. Melanomas express stem cell-associated markers frequently, consistent with the laboratory observation that a single unselected melanoma cell can initiate tumor in in-vivo assays with high frequency. 2. A combination of SOX2 and C-Kit can differentiate melanoma from MPNST with high sensitivity and specificity.

545 Human Endogenous Retrovirus Type K (HERV-K) Envelope Glycoprotein Is Expressed in Melanoma

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Background: Expression of human endogenous retrovirus K (HERV-K) has been reported in melanomas and melanoma cell lines, and enhanced antibody against HERV-K has been shown to be present in sera from melanoma patients. Given its limited expression, it is proposed that HERV-K can potentially serve as a useful marker for melanoma. The aim of this study was to determine whether HERV-K surface glycoprotein is expressed in malignant melanoma (MM) and other melanocytic lesions using HERV-K env-specific 6H5 monoclonal antibody.

Design: Slides from 71 tissues were examined. Staining was performed using monoclonal antibody 6H5, prepared against HERV-K surface glycoprotein, and scored as 0 to 3+.

Results: There was a progressive increase in staining for HERV-K env among the primary lesions. Benign nevi were negative for HERV-K, and dysplastic nevi were mostly negative, with only 1/8 mildly dysplastic nevi (1+) and 2/4 moderately dysplastic nevi (2+) stained, only in the junctional component, with dermal nests negative. There was a significant difference ($p<0.0001$) in staining score between *in situ* melanoma (8 lentigo maligna & 4 malignant melanoma *in situ*) *vs.* the non-nodular MM group (6 lentigo maligna melanoma & 13 superficial spreading MM). There was less staining ($p<0.05$) of the lentigo maligna melanoma sub-group *vs.* the superficial spreading MM sub-group and also *vs.* the nodular melanoma group, but no significant differences between any other invasive melanoma groups. In all MM groups, the staining (mostly 2+ or 3+) extended and involved diffusely the dermal component. Though there was less intense staining in metastatic MM than in unpaired primary melanomas ($p=0.007$), all metastases were positive for HERV-K env glycoprotein.

Tissue	Positive / n	score ± SEM
Benign Nevi	0/10	0
Dysplastic Nevi	3/12	0.42±0.23
In Situ Melanoma	8/12	0.67±0.49
Non-nodular Malignant Melanoma	17/19	1.95±0.91
Nodular Malignant Melanoma	14/14	2.29±0.61
Metastases	4/4	1.25±0.50

Conclusions: HERV-K envelope glycoprotein is detected in melanoma tissues and that expression is associated with melanoma progression. Since activation of latent HERV could be linked with several cancers including melanoma, the expression of immunologically detectable surface glycoprotein might serve as a vaccine target.

Education

546 Use of Whole Slide Digital Images in Residency Education: Utility in Documenting Microscopic Feature Finding Skills

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Background: Whole slide digital imaging has been shown to improve undergraduate educational interaction with histologic and microscopic materials in significant ways. The role of WSDI in post-graduate, post-residency continuing education also appears to be evolving into a significant niche. The use of WSDI in residency level education has been little explored but may offer significant advantages in competency-focused training.

Design: We selected eighteen slides demonstrating a specific microscopic feature that required knowledge of the entity and microscopic locating skills. The slides were scanned as whole slide digital images (WSDI) using an Aperio scanner, and divided into two groups. Eight upper level residents were each given one group of slides as WSDI and one group as traditional glass slides and instructions to find the specified feature and photograph it for verification, using either the Imagescope photocapture tool, or a microscope mounted digital camera. The time required to locate the feature was recorded for each slide but the time needed for photography was not included. Results were stratified by post-graduate year, a subjective technology affinity score, media type (glass vs. WSDI) and case.