Design: A computer-based search of the files of the Department of Pathology, LAC+USC Medical Center was carried out to retrieve breast FNAs performed from 2000 to 2005 which were diagnosed as PBL. Both cytological and surgical slides of these cases were reexamined. A cytological diagnosis of PBL or APBL was used if the findings of the proliferative breast lesion did not fit a more specific category, such as carcinoma, fibroadenoma (FA), or FCC.

Results: 3,934 breast FNAs were performed on palpable breast masses during the perioid. 317 (8.1%) were cytologically diagnosed as PBL with or without atypia. 201 cases (63.4%) had subsequent surgical biopsies. After the cytologic smears were reviewed, 10 cases were diagnosed as FA, 6 as carcinoma, 12 as suspicious for carcinoma, and 1 as unsatisfactory; these 29 cases were excluded from this study. Table 1.

Conclusions: APBL was clinically significant because it was associated with a significant increased likelihood of malignancy compared to PBL without atypia. Most of these malignancies showed hypocellularity and low nuclear grade in the FNA smears. The term atypiain breast cytology denotes uncertainty and increased risk and is not equivalent to the term atypical as ADH used in histology. Most FAs showed some degree of UDH in surgical biopsies, and areas of UDH likely were reflected in most preceding FNA findings of PBL. Most cases in this series lacked one or all major cytologic features of FAs.

Correlation of cytologic and histologic diagnosis in 172 proliferative breast lesions

Histology	No. of cases	APBL	PBL
Malignant	21 (12%)	19 (37%)	2 (2%)
Inv ductal ca	8 (5%)	6 (12%)	2 (2%)
Inv lobular ca	5 (3%)	5 (10%)	0
DCIS	5 (3%)	5 (10%)	0
Other malignant	3 (2%)	3 (6%)	0
Benign	151 (88%)	33 (63%)	118 (98%)
FA	99 (57%)	16 (31%)	83 (69%)
FCC/UDH	12 (7%)	0	12 (10%)
Papilloma	8 (5%)	4 (8%)	4 (3%)
Adenomyoepithelioma	5 (3%)	0	5 (4%)
FCC with UDH	5 (3%)	0	5 (4%)
ADH	3 (2%)	2 (4%)	1 (1%)
ALH	2 (1%)	1 (2%)	1 (1%)
Atypical papilloma	3 (2%)	3 (6%)	0
PT , benign	2 (1%)	0	2 (2%)
Other benign	12 (7%)	7 (11%)	5 (4%)
Total	172	52 (30%)	120 (70%)

388 Clinical Significance of Atypical Glandular Cells in Conventional Smears: A Histologic Follow-Up Study from a Large County Hospital

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Background: This atypical glandular cell (AGC) category remains a diagnostic challenge to both clinicians and cytopathologists. The aim of this study was to determine the rate of AGC and the incidence of clinically significant lesions on subsequent histologic follow-up among a patient population that consists predominantly of low-income and minority women.

Design: Conventional pap smears diagnosed as atypical glandular cells of endocervical origin (AGC-EC), atypical glandular cells of endometrial origin (AGC-EM), and atypical glandular cells not otherwise specified (AGC-NOS) from 2003 to 2005 at the LAC+USC Medical Center were retrieved from the department files. The cases were divided into the following diagnostic categories: ASCUS & AGC, AGC-EM, AGC-EC and AGC-NOS. The histologic diagnoses were correlated with the cytologic diagnoses.

Results: In 64,378 conventional cervicovaginal smears examined during the 3-year study period, AGC was reported in 525 (0.80%) cases, with follow up surgical specimens in 460 (87.6%) of these cases including 38 cone/leep biopsies and 90 hysterectomies. The cyto-histo correlation of these 460 cases is listed in Table 1.

Table 1. Correlation of the AGC Cases with Premalignant or Malignant Lesions in Tissue Biopsies

	AGC & ASCUS	AGC-EM	AGC-NOS	AGC-EC	Total
No. of cases	68 (14.8%)	36 (7.8%)	187 (40.7%)	169 (36.7%)	460
Mean ages	44	50	45	41	44
Sqamous cell lesions	9 (13.2%)	1 (2.8%)	10 (5.3%)	13 (7.7%)	33 (7.2%)
Cervical glandular lesions	0	0	5 (2.7%)	14 (8.3%)	19 (4.1%)
Endometrial lesions	3 (4.4%)	21 (58.3%)	38 (20.3%)	3 (1.8%)	65 (14.1%)
Ovarian lesions	0	1 (2.8%)	4 (2.1%)	1 (0.6%)	6 (1.3%)
Total	12 (17.6%)	23 (63.9%)	56 (29.9%)	30 (17.8%)	121 (26.3%)

Conclusions: In our study population, 26.3% cases with AGC had cancerous or dysplastic squamous or glandular lesions of the exocervix, endocervix, endometrium or ovary, with the most common origin being endometrium. A diagnosis of AGC-EM is the most clinically significant with the highest percentage (63.9%) of women showing premalignant and malignant lesions on subsequent histology. Patients with AGC on Pap smears should undergo immediate intensive diagnostic studies, including colposcopically directed biopsy with endocervical curettage to detect cervical lesions, and endometrial curettage and biopsy to detect endometrial lesions.

Dermatopathology

389 Evaluation of CD10 Expression in Spindle Cell Lesions of the Skin with Emphasis on Atypical Fibroxanthoma

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Background: CD10, a cell surface endopeptidase present in a number of normal cells, carcinomas and sarcomas, has been reported to be a sensitive marker for atypical fibroxanthoma (AFX). However, its expression in various cutaneous spindle cell lesions has not been studied. We evaluated the expression, sensitivity and specificity of CD10 in AFX, in comparison with various spindle cell tumors that may be in its differential diagnosis.

Design: CD10 immunohistochemistry was performed on representative paraffin-embedded sections of 17 AFX, 12 dermatofibrosarcoma protuberans (DFSP), 10 cellular dermatofibromas (CDF), 2 epithelioid dermatofibromas (EDF), 12 spindle cell carcinomas (SCC), 9 spindle cell melanomas (SCM), 7 leiomyosarcomas (LMS) and 2 fibrosarcomas with myofibroblastic differentiation (FMD). Additional 11 malignant fibrous histiocytomas (MFH) were stained for comparison. Diagnoses in all cases were based on morphology, clinical data and immunohistochemistry. Cases were analyzed for pattern of CD10 expression in tumor cells and stroma.

Results: All AFX cases (100%) showed strong and diffuse membranous positivity for CD10 in spindle cells and majority of pleomorphic giant cells (PGC). The latter showed bright membranous and weak cytoplasmic staining. An identical pattern was observed in 10 (100%) CDF (including 4 with monster cells), 8 (73%) MFH, and 2 (100%) FMD cases. The 2 remaining MFH had focal and weak staining only in PGC. Two positive MFH cases contained numerous osteoclastic-like GC, which did not stain for CD10. All (100%) LMS (including 6 that contained PGC), 8 (89%) SCM, and 2 (100%) EDF were negative. One (11%) SCM showed patchy, strong membranous and cytoplasmic staining. CD10 was negative in tumor cells of 11 DFSP (92%), but the surrounding fibroblastic reaction was strongly positive. One case (8%) had diffuse, strong membranous staining. In SCC, 8 cases (67%) were negative and 4 (33%) showed patchy staining of variable intensity within both spindle and PGC.

Conclusions: 1. The sensitivity and specificity of CD10 in AFX were 100% and 67%, respectively. 2. Strong and diffuse CD10 expression was seen within spindle cells and PGC in AFX, CDF and MFH. 3. All LMS, and most DFSP and SCM did not express CD10. However, special care must be taken, since the periphery of some of these tumors may be positive, presumably from reactive fibroblasts around tumor cells. 4. CD10 expression in SCC is variable, possibly because of mixed cell population. Positivity in PGC was observed in a minority of cases.

390 The Evaluation of T Regulatory Foxp3 Expression in Cutaneous T-Cell Lymphocytic Infiltrates

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Background: A growing body of literature exists regarding the role of thymically derived CD4 regulatory T cells (Tregs). The primary function of Tregs is to maintain immunologic tolerance by suppressing self-reactive T cells that have escaped negative selection in the thymus. The development of Tregs depends heavily on the transcription factor Foxp3--the lack of which results in fatal autoimmune lymphoproliferative disease. The role of Tregs in T cell lymphoma has been investigated with conflicting results. This study evaluates the expression and distribution of Foxp3 in various reactive and neoplastic cutaneous T cell disorders.

Design: A variety of T cell lymphocytic infiltrates cases were selected prospectively from both routine and consultative dermatopathology practice and categorized into one of three groups: (1) reactive lymphomatoid; (2) endogenous pre-lymphomatous T cell dyscrasia; and (3) T cell lymphoma. Foxp3 expression was evaluated by standard immunohistochemistry. TCR-beta gene rearrangement studies were performed using multiplex PCR.

Results: Of the 76 cases, 32 (42%) were classified as reactive lymphomatoid, 27 (36%) as T cell dyscrasia, and 17 (22%) as T cell lymphoma. The reactive lymphomatoid category included cases demonstrating lymphoid atypia with potential clonality, but were restricted to those resolving with removal of an identifiable antigenic trigger. The dyscrasia category was represented by pityriasis lichenoides chronica, pigmented purpuric dermatosis, alopecia mucinosa, and large plaque parapsoriasis. The mean Fox3p positivity was 17% overall--22% for the reactive lymphomatoid cases, 15% for the dyscrasias, and 11% for the lymphomas. The most aggressive lymphomas tended to have rare Fox3p cells (<5%), including two subcutaneous panniculitis-like T cell lymphomas, two CD4 NK-like T cell lymphomas, a primary cutaneous CD8 cytotoxic T cell lymphoma and a tumor-stage mycosis fungoides. TCR-beta gene rearrangements studies were available for 66 cases. Of these, monoclonality was seen in 18 (27%), oligoclonality in 20 (30%), and polyclonality in 28 (43%). The mean Fox3p positivity was 13% for the monoclonal cases, 17% for the oligoclonal cases, and 20% for the polyclonal cases.

Conclusions: Foxp3+ T regulatory cells may play a role in controlling the extent of clonal T cell proliferations in the skin with a lack of T regulatory cell function permissive to clonal expansion.

391 Cutaneous Neoplasms with Pagetoid Cells: An Immunohistochemical Study of Mammaglobin Expression

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Background: Mammaglobin belongs to the secretoglobin family of small epithelial secretory proteins and has been characterized as a fairly specific marker for breast carcinoma when employing molecular techniques. Reports on the use of mammaglobin immunohistochemistry are fewer but have shown positive results in up to 80% of cases of breast carcinoma. Neoplasms with pagetoid cells include mammary Paget's disease (MP), extramammary Paget's disease (EMP), melanoma in situ (MIS), and Bowenoid squamous cell carcinoma (BSCC). Our aim was to investigate mammaglobin expression in these lesions, and characterize its utility as an immunohistochemical marker in MP and EMP.

Design: We studied mammaglobin expression using a monoclonal antibody in the following cases: 12 EMP, 5 MP, 5 MIS, and 5 BSCC. All cases of MP were associated with either history of or concurrent breast carcinoma in women.

Results: Immunopositivity was observed in 3 of 12(25%) cases of EMP, and 2 of 5(40%) of MP. Immunostaining was focal in all cases with strength of staining varying from weak to strong. One immunopositive MP also demonstrated staining of the underlying invasive component. Immunopositive cases of EMP were from various sites: perianal (male), scrotum, and vulva. All cases of MIS and BSCC were immunonegative.
Conclusions: These results suggest that immunohistochemical stains for mammaglobin are insufficiently sensitive for MP and EMP. As a result, mammaglobin would not be helpful in differentiating these lesions from other cutaneous neoplasms with pagetoid cells. Lack of expression may be due to various causes including loss of protein expression in cutaneous disease and/or suboptimal antibody clones.

392 Melanocyte Differentiation Antigens (MDAs) and Cancer Testis Antigens (CTAs) – Helpful in the Diagnosis of Spitz Nevi?

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Background: Spitz nevi (SN) are rare lesions which can be difficult to differentiate from malignant melanoma (MM). CTAs such as MAGE and NY-ESO-1 are expressed in various types of malignant tumors and in normal tissues solely in germ cells. No CTA protein expression has been observed in any non-malignant tumor. On the contrary, MDAs such as gp100, Melan-A, and tyrosinase are expressed in normal melanocytes and related to benign and malignant lesions. However, little is known about their diagnostic value in SN.
Design: Paraffin blocks from 46 SN were available for analysis. Immunohistochemistry was done using the following mAbs/to the following CT antigens: mAb MA454/MAGE-A1, M3H67/MAGE-A3, 57B/MAGE-A4, CT7-33/CT7 (MAGE-C1), E978/NY-ESO-1, CT10#5/CT10 and GAGE. For MDA analysis: HMB45/gp100, A103/Melan-A, and T311/tyrosinase.
Results: All 7 anti-CTA mAbs were negative in all 46 SN. In contrast, mAbs HMB45, A103, and T311 were positive in all nevi. While A103 and -to a lesser extent- T311 showed strong immunoreactivity throughout the whole nevi including the dermal component in compound and dermal SN, HMB45 staining was restricted to the junctional region and no to little staining was present in the dermal component.
Conclusions: The differentiation between SN and MM is a frequent diagnostic dilemma in surgical pathology. Diagnosis rests mainly on conventional stains and other techniques are regarded of little help in daily practice. CTAs are novel tumor-associated antigens whose almost exclusive presence in malignant tumors have made them key targets in the immunotherapy of cancer. CTAs are expressed in more than 50% of cutaneous MM. Their absence in this series confirms their restriction to malignant lesions. In contrast, MDAs are expressed in almost all melanocytic lesions to some extent. Interestingly, mAbs A103 and T311 show intense staining in all 46 SN including the dermal component, where present. However, HMB45 staining is almost exclusively present in the junction area. This pattern differs from MM where HMB45 staining is not restricted to the junction region. In conclusion, in dubious lesions the expression of any CTA speaks against Spitz Nevus. In lesions with dermal component, homogeneous staining of A103 and T311 in combination with junctional reactivity of HMB45 suggests Spitz Nevus rather than melanoma.

393 Low-Fat and Fat-Free Spindle Cell Lipoma: A Diagnostic Challenge

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Background: Spindle cell lipomas (SCL) classically occur as subcutaneous masses in the upper trunk/neck of older men and are variably composed of mature fat, cytologically bland, CD34-positive spindled cells, ropey collagen, myxoid matrix, and blood vessels. Some SCL pose diagnostic challenges due to variations in combinational elements. A number of variants have been reported, including SCL with pseudoangiomatous change, composite SCL-hibernoma, and composite SCL/pleomorphic lipoma. We report a series of 34 SCL that posed diagnostic difficulties because of the dearth of fat ("low fat" and "fat-free" SCL).
Design: A review of over 300 consultation cases diagnosed as SCL revealed 34 cases in which fat was noted to be present in <5% of the tumor (n=30) or absent (n=4). All histologic sections and immunohistochemical studies were re-reviewed.
Results: The tumors presented in older men (mean, 56 years; M:F, 11:1) and presented as small (mean, 2.0 cm) circumscribed dermal or subcutaneous masses of the head/neck (n=18), back (n=7), shoulder (n=5), leg (n=2), arm (n=1) or unknown location (n=1). In the majority, referring pathologists considered benign diagnoses, usually benign nerve sheath tumors, but in 4 cases low-grade sarcoma was considered. In only three cases was SCL considered. The tumors were composed of bland spindled cells, often arranged as short parallel bundles, ropey collagen and myxoid matrix. Mature adipocytes were present as isolated cells or rare clusters in 30 cases; 4 cases were devoid of fat. Staghorn branching blood vessels and broad zones of collagen with cracking artifact, as seen in solitary fibrous tumor, were absent. CD34 was diffusely positive (10/11).
Conclusions: "Low-fat" and "fat-free" SCL are rare variants of SCL that may cause significant diagnostic difficulty. A high index of suspicion based on clinical context and identification of other typical features of SCL are diagnostic keys. "Low-fat" and "fat-free" SCL should be distinguished from a variety of other benign and low-grade malignant soft tissue tumors. The histologic features of fat-poor SCL and solitary fibrous tumors overlap to a degree, and it may not always be possible to reliably distinguish them.

394 An Immunohistochemical Panel (Clusterin, CLA, and EMA) Useful in Diagnosing Cutaneous CD30+ Lymphoproliferations

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Background: CD30+ lymphoproliferations (CD30+ LP) in the skin include reactive and clonal processes (primary cutaneous anaplastic large cell lymphoma [C-ALCL] and lymphomatoid papulosis [LyP]) and can be difficult to distinguish. Previous studies have shown expression of clusterin in 40-100% of C-ALCL and EMA in 20-30% of C-ALCL and LyP. Other potentially useful markers, cutaneous lymphocyte antigen (CLA) and JunB, have not been extensively studied. The purpose of this study was to investigate the utility of expression of these antigens in diagnosing C-ALCL, LyP, reactive CD30+ LP, and borderline CD30+ LP.
Design: The 25 cases of CD30+ LP included: 7 C-ALCL; 3 LyP, type C; 8 reactive CD30+ LP; and 7 borderline CD30+ LP (2 ALCL vs. LyP; 5 LyP vs. reactive CD30+ LP). Cases were stained using routine immunohistochemical (IHC) methods with CLA and clusterin (BD Pharmigen, San Diego, CA; 1:200 and 1:200), EMA (Dako, Carpinteria, CA; 1:50), and JunB (Santa Cruz Biotechnology, Santa Cruz, CA; 1:100). Cases with more than 20% large cells staining were scored.
Results: Table 1 summarizes the results of the immunohistochemical stains. Clusterin expression in the large cells of C-ALCL and LyP had a Golgi and cytoplasmic granular distribution. Clusterin positivity was also present in dendritic cells (readily distinguished by cytoplasmic processes), particularly in the reactive CD30+ LP. JunB was positive in all reactive CD30+ LP and all tested C-ALCL; therefore, JunB was not performed on the remaining cases.

Table 1. Antigen Expression in CD30+ LP. Number of cases positive (percentage).

CD30+ LP Category	# of Cases	CLA	Clusterin	EMA	JunB
C-ALCL	7	1/7 (14%)	6/7 (88%)	2/7 (29%)	4/4 (100%)
ALCL vs. LyP	2	1/2 (50%)	1/2 (50%)	1/2 (50%)	N/D
LyP	3	3/3 (100%)	1/3 (33%)	0/3 (0%)	N/D
LyP vs. reactive CD30+ LP	5	5/5 (100%)	0/5 (0%)	0/5 (0%)	N/D
Reactive CD30+ LP	8	6/8 (75%)	0/8 (0%)	0/8 (0%)	8/8 (100%)

N/D = Not Done.

Conclusions: JunB is expressed in C-ALCL and reactive CD30+ LP and, therefore, may be the result of activation of T cells rather than neoplastic transformation. Decreased CLA is suggestive of C-ALCL. Clusterin expression is useful to distinguish C-ALCL and LyP from reactive CD30+ LP. EMA positivity supports a clonal CD30+ LP, but is less sensitive than clusterin.

395 Idiopathic Lymphoplasmacellular Mucositis-Dermatitis

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Background: In 1952, Zoon described a series of patients with dense plasma cell infiltrates in the glans penis. Since then, similar lesions have been described on the external female genitalia and in the airways, for which over 20 designations currently exist. We reviewed 28 such Zoon-like lesions (ZLLs), comparing them with "control" cases of lichen planus (LP), plasmacytoma, and syphilis on the penis, vulva, and oropharynx.
Design: The surgical pathology archive at the University of Virginia was surveyed electronically, to retrieve cases using known aliases of ZLLs, LP, plasmacytoma, and syphilis, affecting mucocutaneous surfaces. Sixty biopsies were obtained. Twenty-eight cases of ZLL, 22 of LP, 8 of plasmacytoma, and 2 of syphilis were evaluated. Twenty-four histologic data points were tabulated in each case, including 12 epidermal features and 12 in the dermis.
Results: Histopathologic findings were similar in all cases of ZLL, regardless of their anatomic sites. They demonstrated superficial cutaneous erosions, basal vacuolar alteration, and lozenge-shaped keratinocytes in the epidermis. The dermis contained a dense inflammatory infiltrate composed predominantly of plasma cells, with scattered neutrophils and lymphocytes. Dense fibrosis was seen in the upper dermis. Epidermal atrophy was apparent most frequently in lesions of the vulva (69%), but it was also noted in others from the oral cavity (43%) and penis (38%). Some differences in ZLL were also noted in regard to other minor features. A majority of vulvar lesions (62%) exhibited dermal vascular prominence but only 38% of those on the penis did so. Extravasation of erythrocytes was seen in 38% of penile lesions and 15% of those on the vulva. No example of ZLL was monotypic or showed accentuation of the epidermal granular layer, "saw-tooth" acanthotic hyperplasia, granulomatous inflammation, or the formation of Civatte bodies, as seen in "control" (non-ZLL) cases.
Conclusions: A uniform nomenclature for ZLLs does not yet exist. Based on the results of this analysis, we suggest that the generic term *idiopathic lymphoplasmacellular mucositis-dermatitis* be considered to encompass the lymphoplasmacellular infiltrates in the skin and mucosal surfaces considered herein. This designation is morphologically descriptive and can be applied regardless of anatomic location.

396 A Case Series and Immunophenotypic Analysis of CK20-/CK7+ Primary Neuroendocrine Carcinoma of the Skin

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Background: Merkel cell carcinoma is a rare and aggressive primary neuroendocrine carcinoma of the skin. This entity is immunohistochemically defined by CK20 expression and with negative CK7 staining. Herein, we present a case series of seven patients with CK20-/CK7+ primary cutaneous neuroendocrine carcinoma.
Design: We analyzed the clinical, pathologic, and immunophenotypic attributes of a series of patients at a large VA and tertiary referral dermatopathology service. The clinical data was obtained by chart review and all diagnoses were confirmed by a

Board Certified dermatopathologist; CK7 prediluted Dako, CK20 prediluted Dako, synaptophysin prediluted Dako, and TTF-1 prediluted Dako. The results were interpreted and analyzed by a single dermatopathologist, and a minimum of 10% staining of the tumor volume was considered positive.
Results: The patients consisted of five males and two females with a mean age of 65, and all patients were Caucasian. All the neoplasms were located in the head/neck (4) and the extremities (3). The histologic features showed a diffuse dermal population of epitheliod cells possessing enlarged nuclei containing a salt and pepper chromatin pattern. Each of the neoplasms showed a brisk mitotic rate with focal necrosis. All of the seven cases showed diffuse cytoplasmic staining for CK7 and positive staining for synaptophysin. CK20 and TTF-1 staining was negative. Clinical work-up for metastatic disease including CT scan of the thorax was negative. Clinical follow-up is limited, with all patients alive with no evidence of metastatic disease at 6 months of follow-up.
Conclusions: Herein we have presented a hitherto reported group of patients with CK7+/CK20- primary neuroendocrine carcinoma of the skin. Although the findings are preliminary, the clinical and histologic parameters of these patients is similar to conventional Merkel cell carcinoma. The pathogenic significance of these findings is yet to be determined.

397 Diagnostic Value of CD23 in Merkel Cell Carcinoma and Small Cell Carcinoma

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Background: The distinction between Merkel cell carcinoma (MCC) and metastatic small cell carcinoma (SCC) can be a diagnostic challenge. During our daily practice, we observed that CD23 labels Merkel cells in normal skin. In this study, we examined the staining profile of CD23 in MCC and assessed its usefulness in distinguishing MCC from SCC.
Design: Immunohistochemical staining of CD23 (clone BU38) was performed in 23 MCCs (21 cutaneous tumors and 2 lymph node metastases) and 18 SCCs (12 pulmonary, 4 non-pulmonary and 2 distant metastases of pulmonary origin). The amount of staining was quantitatively assessed as 0%, <1%, 1-10%, 10-50%, and >50%. The pattern of staining was categorized as cytoplasmic or perinuclear dot-like. A Chi-squared test was used to analyze the statistical significance of the findings.
Results: CD23 positivity was present in all but one (22/23, 96%) of MCCs and in the majority (16/18, 91%) of SCCs . Although most positive cases of both tumors demonstrated strong and diffuse CD23 staining, there was a significant difference in the staining pattern between MCCs and SCCs (P<0.02). In MCCs, 14 of 22 (64%) demonstrated a predominance of perinuclear dot-like staining pattern, similar to that of cytokeratin 20. The prevalent pattern in the remaining 8 cases (36%) was cytoplasmic, with 4 of 8 having focal perinuclear dot-like staining. In comparison, all (16/16) SCCs (pulmonary and non-pulmonary) showed a diffuse cytoplasmic staining. Among the small number of metastatic cases tested, a staining difference was not identified between metastatic vs. primary tumors of MCC or SCC.
Conclusions: CD23 labels the majority (18/22, 82%) of MCCs with a diffuse or focal perinuclear dot-like staining. This pattern is not observed in SCCs. The findings suggest that CD23 could serve as a useful ancillary tool in differentiating MCC from SCC. To our knowledge, this is the first study to demonstrate the utility of CD23 in the immunohistochemical workup of MCC.

CD 23 Staining in Merkel Cell Carcinoma and Small Cell Carcinoma

	Merkel Cell Carcinoma	Small Cell Carcinoma
Positive		
perinuclear dot-like	14	0
cytoplasmic	8*	16
Negative	1	2
Total	23	18

* 4 of 8 cases had focal perinuclear dot-like staining

398 D2-40 Is Another Useful Marker in Differentiating Dermatofibroma from Dermatofibrosarcoma Protuberans

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Background: Differentiating dermatofibroma (DF) from dermatofibrosarcoma protuberans (DFSP) can be difficult on superficial biospy. Typically, DF is positive for Factor XIIIa, and negative for CD34; while DFSP is negative for Factor XIII and positive for CD34. The panel of Factor XIIIa and CD34 has been used widely in the diagnosis of DF vs. DFSP. However, small numbers of DF might be negative for Factor XIIIa and sometimes even with increased CD34 reactivity making the differential diagnosis of DF vs. DFSP difficult. Podoplanin is known to express in lymphatic endothelial cells, mesothelial cells and skin adnexal cells, and the tumors arising from these cells. In addition, its expression is also seen in certain mesenchymal cells such as certain dendritic cells in superficial dermis. We used D2-40 a monoclonal antibody against podoplanin in this study, to assess its diagnostic utility in the diagnosis of DF vs. DFSP.
Design: Immunohistochemical assays were performed on 5 µm-thick formalin-fixed paraffin-embedded sections. Heat induced epitope retrieval was performed before incubation of primary antibody (D2-40, Signet Laboratories, Dedham, MA). We examined the expression of D2-40 in 12 DF, 23 DFSP and 18 neurofibroma (NF). Most of the DF and DFSP were evaluated for Factor XIIIa and CD34 and all NF for S100 at the time of the diagnosis. D2-40 immunoreactivity was evaluated semiquantitatively in lesional cells on a two-tiered scale based on the percentage of the staining (2+ >60%, 1+ <60%).
Results: In 12 DF, D2-40 is positive in all cases (100%); strongly (2+) in 11 (92%), and 1+ in one (8%). In 23 DFSP, D2-40 is focally positive (1+) in 10 cases (43%), and negative in 13 cases (57%). In NF, D2-40 is positive in 6 cases (32%), 1 strongly (2+, 5%) and 5 focally (1+, 27%), and negative in 12 cases (68%). In 3 DF cases, the Factor XIIIa was weak or negative but D2-40 was positive.
Conclusions: D2-40 immunoreactivity is detected in all DF but only in small numbers of DFSP and NF. D2-40 immunoreactivity is strong and diffuse in almost all the DF in contrast to weak and focal in those of DFSP and NF exhibiting D2-40 reactivity. D2-40 can be useful in differentiating DF from DFSP and NF even when Factor XIIIa was negative in DF cases. Adding D2-40 to the current panel of Factor XIIIa and CD34 for differentiating DF vs. DFSP can further improve the diagnostic certainty of the immunohistochemical panel in cases with ambiguous CD34 and Factor XIIIa.

399 Targeting Bfl-1 Expression Inhibits Melanoma Cell Survival

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Background: The bcl-2-related gene expressed in fetal liver (Bfl-1/BCL2A1/A1) is a bcl-2 family member preferentially expressed in bone marrow, spleen, and lymphoid tissues, as well as tumor-infiltrating inflammatory cells. BFL-1 binds to Bid and truncated Bid(tBid), preventing the mitochondrial membrane permeability change necessary for release of pro-apoptotic factors, thus inhibiting the intrinsic pathway of cell death. Recently it was reported that melanoma cells treated with gamma-interferon resulted in inhibition of cell growth and concomitant down-regulation of Bfl-1 as assessed by expression profiling. In the present study, we investigated the effects of targeting Bfl-1 expression by anti-sense technique.
Design: Melanoma cells A375 and SK-mel-1 and melanocyte MC-LP (Cascade Biologics) were used. Bfl-1 expression was assessed by real-time quantitative PCR. Bfl-1 sense and antisense oligonucleotide (5'-CCAAATTCACAGTCTGTCAT-3') was designed and synthesized (Invitrogen). Transfection was done by using the Lipofectamine protocol (Invitrogen). Cell survival was measured by the MTT method every 12 hours for 48 hours after transfection with or without UV-irradiation. Fluorescence indicator Calcein-AM (Sigma) and its quencher CoCl2 were used to monitor changes in mitochondrial membrane permeability.
Results: The Bfl-1 mRNA copy numbers increased 4.3-27 fold in the melanoma cells relative to that in normal melanocyte MC-LP, as measured by quantitative RT-PCR. Bfl-1 antisense oligonucleotide treatment resulted in significant downregulation of Bfl-1 expression (>95%), most prominent within 24-36 hours of transfection. Sk-Mel-1 cell survival rate reduced by 25% (without UV-irradiation) to 35% (with UV-irradiation). Cell survival of A375 cells did not show significant change in the absence of UV-irradiation but reduced by 25% with UV-irradiation. Both SK-mel-1 and A375 cells demonstrated changes in mitochondrial membrane permeability after Bfl-1 antisense oligonucleotide treatment, particularly when combined with UV-irradiation.
Conclusions: Bfl-1 antisense oligonucleotide treatment of melanoma cells could downregulate Bfl-1 expression, inhibit cell growth, and potentiate sensitivity to UV-irradiation, which is associated with changes in mitochondrial membrane permeability.

400 Expression of Stem Cell Biomarker OCT4 in Cutaneous Neoplasia

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Background: The OCT4 transcription factor is a marker of pluripotency and is present in embryonic stem cells, primordial germ cells and several neoplasms including seminoma, dysgerminoma and embryonal carcinoma. Recently immunohistochemical expression of OCT4 protein has been described in the cells within the basal layer of normal human and canine epidermis. We have examined a series of basal cell carcinomas and adnexal tumors of related histiogenesis, in an effort to corroborate the above findings and to assess for expression of OCT4 protein in neoplasia of the infundibulo-apocrine-sebaceous unit.
Design: 115 cutaneous specimens were examined for OCT4 expression by immunohistochemistry. Assessment of adjacent or overlying benign epidermis and follicular epithelium for OCT4 expression was also performed.
Results: We analyzed OCT4 expression in 115 cutaneous specimens including 26 basal cell carcinomas, 12 benign follicular tumors (10 trichoepitheliomas and 2 trichoblastomas), 10 benign apocrine tumors, 12 sebaceous hyperplasia lesions, 10 sebaceous adenomas, 4 sebaceous carcinomas and 13 nevi sebacei of Jadassohn, 8 squamous cell carcinomas (including one spindle-cell squamous cell carcinoma), 8 compound melanocytic nevi, 5 Mërkel cell carcinomas, 3 pilar cysts, one scar, two non-specific, mild superficial perivascular dermatitis specimens, and one non-scaring alopecia. All 115 specimens examined were negative for OCT4 expression as was adjacent or overlying epidermis and follicular epithelium including the bulge region.
Conclusions: In contrast to previous studies, our data indicate that the OCT4 expression is not retained in cutaneous neoplasms derived from basal epidermis or related adnexal neoplasms, including lesions of the scalp.

401 Expression of S100 Protein Subtypes in Desmoplastic Melanoma, Neurofibroma and Schwannoma

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Background: Desmoplastic melanoma (DM) is a variant of malignant melanoma characterized by spindle cells and prominent fibrous stroma. It may simulate sclerosing melanocytic nevi as well as various benign and malignant non-melanocytic lesions, including benign peripheral nerve sheath tumors (BPNSTs). The latter case is diagnostically challenging since many cases of DM only show polyclonal S100 protein expression but no reaction to melanocytic markers. The S100 family of proteins consists of over 20 members that share a common calcium binding motif. We report on various S100 subtypes in desmoplastic melanoma and BPNSTs in order to identify and evaluate differential expression.

Design: 23 cases of cutaneous DM, and 21 cases of BPNSTs including 11 cases of Schwannoma and 10 cases of neurofibroma were studied. Immunostains for S100A1, S100A2, S100A4, S100A6, polyclonal S100 (pS100), HMB-45, Melan-A, tyrosinase, PNL2 and microphthalmia transcription factor (MITF) were performed using tissue microarrays. The staining was graded to focal (<50%) and diffuse (>50%).
Results: Two-third of the cases of DM showed diffuse reaction to S100A1 whereas one-third of BPNSTs showed only focal reaction with the remainder being negative. S100A2 was negative in both groups. S100A4 was diffusely expressed in a majority of DM cases, but its expression was variable in BPNSTs. S100A6 expression was diffuse in DM and Schwannomas, but variable in neurofibromas. pS100 was diffusely stained in all the cases of DM and BPNSTs. Each melanocytic marker stained only in a small subset of cases of DMs, to an extent ranging from 9% in Melan-A to 21% in MITF. They were completely negative in Schwannomas and neurofibromas as expected.
Conclusions: A majority of DMs were negative for melanoma markers as previously reported. Diffuse expression of S100A1 was only seen in DM but not in BPNSTs. This may be diagnostically helpful to distinguish between the two entities.

402 PAX-5 and CD15 Can Distinguish between Type A Lymphomatoid Papulosis and Cutaneous Involvement by Hodgkin Lymphoma

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Background: Cutaneous involvement by primary or systemic Hodgkin lymphoma (HL) is a rare event, occurring in approximately 0.5-3% of HL patients with long-term follow-up. In contrast, lymphomatoid papulosis (LyP), a cutaneous lymphoproliferative disorder, commonly presents in association with cutaneous and systemic lymphomas, including HL. Cutaneous HL and LyP may appear similar clinically, presenting as a papular or nodular eruption involving the trunk. Furthermore, there is histologic and immunophenotypic overlap between these two entities with both displaying numerous large, pleomorphic CD30+ cells set within a polymorphous inflammatory background. The distinction between these two lesions is clinically important as cutaneous involvement by HL carries a dismal prognosis compared to LyP which is generally indolent.
Design: Twenty cases of type A LyP were identified and retrieved from the Stanford pathology archives and were compared with 8 previously characterized cases of cutaneous HL. We stained each case with antibodies to CD15 (Ventana, pre-diluted), CD30 (Dako, 1:20), and PAX-5 (BD Transduction Laboratories, 1:100). Clinical information was obtained from review of the patients' medical records.
Results: Our study set comprises 11 males and 9 females diagnosed with type A LyP who ranged in age from 13-65 years (average=47 years). Four patients had co-existing mycosis fungoides and one had a history of cutaneous anaplastic large cell lymphoma. Interestingly, one patient was diagnosed with systemic HL at time of presentation with LyP. Three men and five women comprised the cutaneous HL group, and they ranged in age from 44-79 years (average=56 years). Of this group, four patients presented with primary cutaneous HL and three with secondary involvement by systemic HL. Immunophenotypic data are displayed in Table 1.

Immunohistochemical Findings in Lymphomatoid Papulosis and Cutaneous Hodgkin Lymphoma

Entity	CD30	CD15	PAX-5
Lymphomatoid papulosis	20/20	0/20	0/19
Cutaneous Hodgkin lymphoma	8/8	5/8	6/6

All but one case of HL stained with CD15 and/or PAX-5. In contrast, both CD15 and PAX-5 were negative in all cases of LyP.
Conclusions: To our knowledge, this is the first reported series showing the utility of CD15 and PAX-5 in distinguishing cutaneous HL from LyP, which is a distinction of significant clinical importance.

403 Sterile Neutrophilic Lobular Panniculitis: A Novel Subcutaneous Expression of the Acute Infectious ID Reaction

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Background: Panniculitis can be categorized as lobular or septal. Septal encompasses lupus profundus, atypical lymphocytic lobular panniculitis, erythema induratum, and subcutaneous Sweet's syndrome, while lobular includes erythema nodosum. Panniculitis may be a sign of systemic disease and/or represent a modified immune response to hematogenously disseminated antigen.
Design: We describe 7 cases of sterile neutrophilic dominant lobular panniculitis that represented an id reaction to various infectious stimuli, excluding mycobacterium.
Results: The patient population included 2 males and 5 females, ages 6-62, who presented with sudden tender non-ulcerated nodules and plaques on the lower extremities. One male also had upper extremity lesions. All patients had nontuberculous infectious triggers; among the underlying infectious based etiologies were two cases of recurrent sinusitis, a breast abscess, a dental abscess, impetigo, cellulitis, and viral pharyngitis. The lesions subsided with antibiotic treatment and/or abscess drainage; no case recurred. Four patients had an atopic diathesis, one demonstrated polyclonalhypergammaglobulinemia, one had circulating cold agglutinins, and one had primary anti-phospholipid antibody syndrome. All biopsies revealed a dominating neutrophilic infiltrate admixed with mononuclear cells, 5 with subcutaneous microabscess formation and concomitant variable fat necrosis. 3 cases had extravascular granulomatous infiltrates; 3 had thrombotic microangiopathy affecting subcutaneous lobular capillaries; 3 had necrotizing vasculitis involving venules and arterioles at the dermal subcutaneous interface. Most cases showed concomitant interface dermatitis. In all cases, special stains evaluating microbial pathogens were negative.
Conclusions: We propose the term acute infectious id panniculitis to describe those cases of neutrophilic lobular panniculitis triggered by nontuberculous infectious stimuli; a thrombotic microangiopathy and interface dermatitis are additional morphologic clues. This course may be self-limited. Predisposing microvascular co-factors promoting the mural entrapment of immune complexes such as transient infection associated hyperviscosity and/or procoagulant state may be pathogenetically important; recognizing this entity may circumvent the need for an exhaustive evaluation for other cases of neutrophilic lobular panniculitis.

404 Cutaneous Lymphocyte-Associated Antigen Expression in Cutaneous B- and T-Cell Lymphomas

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Background: Cutaneous lymphocyte-associated antigen (CLA) is expressed in resident cutaneous T lymphocytes, high endothelial venules, peripheral monocytes, granulocytes, and memory T cells. It has been postulated to be an important factor in homing lymphocytes to the skin and demonstrated in a variety of T cell malignancies, namely those originating in the skin.
Design: We investigated the CLA presence of 51 cutaneous lymphoid neoplasms of T and B cell origin.
Results: The vast majority of B cell malignancies were negative including marginal zone lymphoma, primary and secondary follicle center cell lymphoma, mantle cell lymphoma and diffuse large cell B cell lymphoma; however, dot-like positivity was seen in 3 cases of primary cutaneous B cell lymphoblastic lymphoma and one case of marginal zone lymphoma. There was greater heterogeneity of expression in T cell malignancies. All cases of mycosis fungoides, chronic smoldering variant of adult T cell leukemia/lymphoma, CD30 positive lymphoproliferative disease including lymphomatoid papulosis and anaplastic large cell lymphoma, primary cutaneous aggressive cytotoxic CD8 lymphoma, and primary cutaneous pleomorphic small and medium sized pleomorphic T cell lymphoma were extensively positive. However, 2 cases of tumor stage mycosis fungoides manifested striking reduction in staining. There was discernible yet substantially diminished staining with aggressive forms of primary and secondary cutaneous T cell lymphoma including CD30 negative large cell T cell lymphoma, NK like T cell lymphoma, panniculitis-like T cell lymphoma, T cell lymphoblastic lymphoma, and T cell prolymphocytic leukemia.
Conclusions: While CLA expression may play a role in homing T cell neoplasms to the skin, its expression is not exclusive to T cells; there may be a role in tumors of B cell origin, namely primary cutaneous B cell lymphoblastic lymphoma, a tumor with a known proclivity to involve the skin. Conversely the loss of CLA expression in a primary cutaneous T cell lymphoma that is normally positive for this marker may be a feature of disease progression. Although its expression does not specifically confer a clinical course of indolence, as it can be seen in primary cutaneous aggressive cytotoxic CD8 lymphoma, a diminished expression pattern is seen more characteristically in those primary and/or secondary T cell lymphomas that are intermediate grade and aggressive forms of lymphoid neoplasia.

405 WT1 Expression in Malignant Melanoma Is Associated with Aggressive Behavior

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Background: Distinguishing benign from malignant melanocytic lesions can be problematic. The transcription factor Wilms tumor 1 (WT1) has previously been reported to be over expressed in melanoma cell lines in vertical growth phase but not in cell lines in radial growth phase or in benign nevi. WT1 has also been suggested as a diagnostic marker to distinguish malignant melanomas from benign nevi. This study explores WT1 expression in a variety of melanocytic lesions to determine its utility as a marker of malignancy and predictor of clinical aggressiveness.
Design: Twenty-nine cases of malignant melanoma, including 8 nodular, 2 superficial spreading, 1 acral lentiginous, 1 desmoplastic, 1 lentigo maligna with invasion, 11 in-situ melanomas, and 5 metastatic lesions (with no available previous diagnosis) were studied. Sites of metastases included lymph node (2), lung, intestines, and dermis. Lymph node metastasis from 3 of the nodular melanomas as well as 31 conventional nevi, 11 dysplastic nevi, and 2 Spitz nevi were also included in the study. Immunohistochemical staining for WT1 was performed using a commercially available monoclonal antibody on paraffin-embedded formalin-fixed tissue. Expression was graded semi quantitatively based on extent and intensity of staining.
Results: WT1 was expressed in 11 of 29 malignant melanomas (38%), including all 8 nodular melanomas and 3 of 5 metastatic melanomas with no available previous diagnosis. Staining was usually cytoplasmic, intense, and diffuse. None of the other melanoma subtypes expressed WT1. All 11 WT1 positive cases were invasive melanomas, while only 7 of 18 (39%) of WT1 negative cases were invasive ($p<0.001$). Furthermore, 8 of 11 (73%) WT1 positive cases developed metastases, while 5 of 18 (28%) WT1 negative cases had metastases ($p<0.02$). The metastases from the WT1-expressing melanomas retained WT1 expression in all 3 cases examined. WT1 was focally expressed in 1 of 11 (9%) dysplastic nevi, 1 of 2 Spitz nevi (50%), and 4 of 31 (13%) of conventional nevi.
Conclusions: Diffuse strong expression of WT-1 appears specific for melanoma, specifically the nodular subtype, compared with other melanocytic tumors. WT1 expression is associated with more aggressive behavior, including invasion and metastases. Further studies are warranted to fully evaluate WT1 as a prognostic marker and therapeutic target in malignant melanoma.

406 Dermatofibrosarcoma Protuberans Treated with Wide Local Excision: Prognostic Significance of Clinicopathologic Variables

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Background: Dermatofibrosarcoma protuberans (DFSP) is a rare,cutaneous low grade sarcoma which is characterized by tendency to local recurrence and rare distant metastasis. Local control of the disease can usually be achieved by wide local excision but some patients still develop local recurrences. The aim of this study was to investigate the correlation between common well-known clinicopathologic factors and survival in a large series of DFSP patients from a single cancer center.

Design: The tissue sections and medical records of 122 patients with primary DFSP from were selected and reviewed from the files of the Department of Pathology at the University of Texas- MD Anderson Cancer Center between 1976 and 2005. Clinicopathologic features were analyzed and compared with recurrence free survival (RFS) and overall survival (OS) were determined.

Results: There were 63 female and 59 male patients with a median age of 43 years. Fibrosarcomatous change was detected in 24 (20.9%) patients. During follow-up, 35 of the 122 patients (28.6 %) developed local recurrence, with a RFS rate at 5 years of 65,3%. Fibrosarcomatous change, mitotic count, metastasis and acral tumors were significantly associated with shorter RFS. On multivariate analysis, tumor location, and fibrosarcomatous change remained significant. Five year OS was 93.5% and median OS was 47 months. On univariate analysis, mitotic count per mm^2, presence of necrosis and metastasis were significantly associated with lower OS. On multivariate analysis, only metastasis remained significantly associated with OS. A positive surgical margin was not associated with RFS and OS.

Conclusions: According to our data, fibrosarcomatous change, high mitotic count and acral location are associated with increased recurrence rate. Therefore, a wider resection should probably considered in such cases. With the exception of metastases none of the analyzed factors correlated with decreased OS in a multivariate analysis.

407 ErBb Receptor Family Expression in Melanocytic Lesions

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Background: The erbB family of receptors is involved in tumor formation and progression. Expression of erbB1, 2, and 3 has been variously reported in nevi and melanomas; erbB4 investigation in this context has been limited. This study examined the expression of all four erbB receptors in malignant melanoma (MM), dysplastic nevi (DN) and common nevi (CN).

Design: Formalin-fixed, paraffin-embedded tissues of 100 primary MM, 27 DN, and 23 CN were immunostained with antibodies against erbB1 (Zymed), 2 (Dako), 3 and 4 (Santa Cruz). The percentage and intensity of immunostaining were measured semiquantitatively and analyzed statistically.

Results: ErbB4 was most widespread in CN (96%), then DN (87%) and MM (66%) ($p<0.0001$). Corresponding values for erbB3 were 96%, 65% and 85% and for erbB2 37%, 57% and 30% (NS). ErbB1 expression was negligible. ErbB3 was most intense in CN, then DN and MM ($p<0.0001$) and more often membranous in CN than DN; in MM, staining was predominantly cytoplasmic ($p<0.0001$). Staining patterns were regular in CN and DN but haphazard in MM. Spatial overlap of the receptor stains was prominent in CN. Thicker MMs had more widespread positivity for erbB2 and 3 in vertical (VGP) than radial growth phases (RGP) ($p=0.02$, borderline $p=0.07$, respectively) and in dermal than epidermal components (borderline $p=0.07$, $p=0.04$, respectively); lesions with higher mitotic rate had more widespread erbB2 expression in the VGP ($p=0.003$) and more intense expression of erbB3 and 4 in the dermal component ($p=0.023$, $p=0.019$, respectively). Lymphocytic infiltrates were heavier in MM with more widespread erbB2 staining ($p=0.03$).

Conclusions: This study revealed erbB2, 3 and 4 receptor expression in all subtypes of melanocytic lesions, but with quantitative and qualitative differences. There was higher positivity of erbB3 and 4 in nevi than melanomas, and in CN than in DN, indicating loss of receptor expression with tumor progression. Nevi also had a more mature pattern of expression than MM and more prominent membranous erbB3 staining. In MM, despite the generally lesser erbB positivity, the association of negative prognostic factors (thickness, mitotic rate) with more prominent erbB expression in the VGP and dermal component may point to mutations in these receptors. The presence of heavier lymphocytic infiltrates in MM with stronger expression of erbB2 could be due to its immunogenicity. The complex patterns of erbB receptor expression in melanocytic lesions warrant further investigation.

408 Neurothekeoma: An Analysis of 178 Tumors with Detailed Immunohistochemical Data and Long-Term Patient Follow-Up Information

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Background: This report describes the clinicopathologic findings in 176 patients (pts) who presented with 178 tumors currently classified as neurothekeomas (NTK).

Design: Archival cases (n=345), accessioned between 1970-99 and coded as NTK or nerve sheath myxoma (NSM), were screened using updated criteria and IHC.

Results: Our study group included 64 males & 112 females, 20 mos-85 yrs old at the time of their first surgical procedure (median:17 yrs); 24% of pts were ≤10 yrs old and only 20% of pts were ≥ 30 yrs of age at initial diagnosis. The pts typically presented with a solitary, asymptomatic mass, 0.3-2.0 cm in size. One pt had multiple tumors. More than 75% of the lesions involved the head (n=63), upper extremities (n=44), and shoulder girdle (n=27) regions. Histologically, the lesions involved the dermis +/- subcutis, and they formed multinodular masses with varying amounts of myxoid matrix. Based on matrix content, the tumors were subclassified as cellular (n=63), mixed (n=67) or myxoid (n=48). All cases had spindled and epithelioid mononuclear neoplastic cells with relatively abundant cytoplasm and indistinct cell borders, and many also had some multinucleated tumor cells. The lesional cells had a strong tendency for whorled growth, and focal fascicular growth was also present. Nuclear atypia was minimal or mild in 135 cases, at least focally moderate in 41 cases, and focally marked in 2 cases. Mitotic counts ranged from 0-124 mit. figs.(MF)/25 wide high power fields (WHPF) (median:4 MF/25 WHPF); 25 lesions had >10 MF/25 WHPF, and 16 had atypical MF. Osteoclast-like giant cells were noted in 39% of cases. Immunoreactivity was typically present for VIM, NKI/C3, CD10, MITF, and PGP9.5, +/- focal reactivity SM actin and CD68. The tumors were (-) for S100 protein, GFAP, and Melan A. Most cases had (+) margins. Complete follow-up (FU) was available for 71 pts (40.3%)[median interval:17 yrs 9 mos], and incomplete FU was available for 14 additional pts [median interval:5 mos]. Regrowth of tumor was reported in 13 pts (1 w/ 2 recurrences). However, due to a tendency for clinicians to send us cases with a complex clinical course, this is likely an overestimation of the true recurrence rate.

Conclusions: NTKs are distinct from true NSMs. They have a peak incidence in the 2nd decade, a female predominance, a predilection for upper body regions, and a relatively low recurrence rate. A fibroblastic origin is proposed.

409 CD10 Immunoexpression in Cutaneous Spindle Cell Neoplasms

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Background: Atypical fibroxanthoma (AFX), spindle cell squamous cell carcinoma (SCC), and spindled melanocytic neoplasms can have a similar appearance on H&E staining; however, distinguishing between these cutaneous neoplasms is essential for prognosis and treatment. Immunohistochemical (IHC) stains for cytokeratin (CK) and S100 are positive in spindle cell SCC and melanocytic neoplasms, respectively, and help with the diagnostic dilemma. CD10, a cell-surface endopeptidase, is purported to be a useful marker for AFX. We studied the immunoexpression of these three antibodies in the above mentioned cutaneous spindle cell neoplasms to assess the utility of CD10 staining in the differential diagnosis.

Design: 53 cases of cutaneous spindle cell neoplasms were identified from the pathology files. The cases consisted of 26 AFX, 9 spindle cell SCC, and 18 melanocytic neoplasms. The diagnoses were established by four pathologists (DLG, MP, CHP, BEFJ). Immunohistochemical stains for CK 5/6, S100, vimentin, and CD10 were performed using conventional automated immunohistochemistry with appropriately reactive controls. The stains were scored with consensus by 3 pathologists (DLG, CHP, BEFJ) blinded to the diagnosis. Positive staining was reported with immunoreactivity in at least 10% of neoplastic cells.

Results: The results are outlined in Table 1. 24 of 26 AFX cases (92%) showed positive staining with CD10. 8 of 9 spindle cell SCC (89%) and 2 of 18 melanocytic neoplasms (11%) showed positive staining for CD10. Vimentin stained the majority of cases (>89%) in each of the neoplasms.

Conclusions: As previously described, vimentin is a non-specific stain in cutaneous spindle cell neoplasms. CD10 is a sensitive stain for AFX (92% sensitivity) but is also relatively non-specific (63% specificity), staining the majority of spindle cell SCC and some melanocytic neoplasms. Although negative staining for CD10 may help eliminate AFX from the differential diagnosis, it has little utility in distinguishing these cutaneous spindle cell neoplasms.

Table 1. CK 5/6, S100, Vimentin, and CD10 immunostaining in cutaneous spindle cell neoplasms.

Cutaneous spindle cell neoplasm	# Cases	No. (%) CK 5/6 +	No. (%) S100 +	No. (%) Vimentin +	No. (%) CD10 +
Atypical Fibroxanthoma	26	0 (0)	0 (0)	25 (96)	24 (92)
Spindle Cell SCC	9	9 (100)	0 (0)	8 (89)	8 (89)
Melanocytic	18	0 (0)	17 (94)	17 (94)	2 (11)

410 Suppression of the Tumorigenicity of B-16V Melanoma Cells Transfecting with pHM6 Vector Carrying Mouse Lysozyme Gene

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Background: Lysozyme (muramidase) is a natural antibacterial agent existing in many body fluids. Data from previous studies show that lysozyme also has analgesic, anti-tumoral, anti-metastatic, anti-inflammatory, and immunomodulatory activities.

Design: The aim of this study is to evaluate the effects of lysozyme on the tumorigenicity of B-16V melanoma cells. For this purpose; recombinant pHM6 vector harboring mouse lysozyme gene (pHM6mLys) was constructed after a series of applications including mRNA isolation, RT-PCR, restriction digestions and ligation to the mammalian plasmid vector. B-16V melanoma cells were transfected with endotoxin free plasmid DNA (pHM6 and pHM6mLys)/liposome complexes and transfectants were selected in media containing Geneticin (G 418). The cells were injected subcutaneously to C57BL/6 inbred mice ($1x10^5$ cells/mouse). Tumor free animals challenged with wild type B-16V cells. In addition, pHM6 and pHM6mLys plasmids were injected into the tumor generated by B-16V cells. The tumors were evaluated for the differences in their volume and weights and also for the tissue necrosis, mitotic activity and tumor infiltration lymphocytes ($CD4^+$ and $CD8^+$ T cells) in tissue sections from these tumors. The results were evaluated statistically.

Results: There were no morphological and growth condition differences between wild type and transfected B-16V clones. In all 60 mice injected with wild type B-16V cells (30 mice) and B-16VpHM6 cells (30 mice) visible tumors were developed after 8-12 days of injection and continued to grow. No tumors were developed in 15/29 (51.7%) of the mice injected with B-16VpHMmLys. Findings were regarded as significant if p values were ≤ 0.05. None of 7 mice challenged with wild type B-16V melanoma were

developed tumors until the end of experimental days 60. Tumor volume and weight were smaller, and there was more tumor necrosis, high mitotic activity and more $CD8^+$ T cell infiltration in pHM6mLys injected tumors than controls (B-16V and B-16V injected with pHM6).

Conclusions: These findings show that lysozyme has an important suppression on the tumorigenicity of B-16V melanoma cells, and surviving tumor free mice developed protective immunity against wild type B-16V cells. *This study was supported by Cukurova University, Academic Research Projects Unit (TF.2002.D2).

411 CD25 Expression in Cutaneous Mast Cells from Adult Patients Presenting with Urticaria Pigmentosa Is Predictive of Systemic Mastocytosis

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Background: Mast cell disease (MCD) comprises a heterogeneous group of disorders defined by accumulation of mast cells in one or more organ systems. MCD includes cutaneous mastocytosis (CM), most commonly the maculopapular type/urticaria pigmentosa (UP), and systemic mastocytosis (SM), characterized by multifocal lesions in bone marrow and/or visceral organs. Whereas pediatric CM often resolves spontaneously by puberty, adult-onset UP shows no such tendency, and a significant subset of patients initially diagnosed with UP are found to have SM upon additional investigation. Recently, expression of CD25 on bone marrow mast cells has been shown to be specific for SM. However, CD25 expression in CM has not been examined. The purpose of this study was to evaluate the clinicopathologic features of adult patients with CM, and to determine whether immunohistochemical detection of CD25 in skin biopsies can be used to predict SM.

Design: Skin biopsies from 30 patients (13M/17F; mean age: 48 yrs, range: 17-80) with an initial diagnosis of CM were evaluated. All cases were immunostained for c-kit (CD117), mast cell tryptase, and CD25. Clinical details and follow-up (mean: 6 yrs; range: 3 mos-19 yrs) were obtained from hospital medical records.

Results: In 17/30 cases, mast cells were predominantly perivascular in distribution; 13/30 showed a mixed interstitial and perivascular pattern, including 2 with confluent sheets of mast cells in the papillary and upper reticular dermis. Mast cell density ranged from 22-482 per HPF (mean 101). Mast cells were round in 10/30 cases, and mixed round and spindle-shaped in 20/30. On further investigation, 9 patients (30%) were found to have SM, 8 within 9 mos of skin biopsy, and 1 after 9 yrs of follow-up. No histologic features correlated with SM. Interestingly, cutaneous mast cells from all 9 patients with SM (100%) were immunoreactive for CD25, compared to only 5 of 21 (24%) patients with limited CM ($p<0.001$). Of the latter 5 patients, 2 had negative bone marrow biopsies, and 2 had greater than 6 yrs of follow-up.

Conclusions: Immunohistochemical detection of CD25 in cutaneous mast cells from adult patients presenting with UP is highly predictive of SM, although mast cells in occasional patients with limited skin involvement also express CD25. CD25 immunohistochemistry may help stratify adult UP patients for further evaluation and management.

412 Overexpression of Focal Adhesion Kinase in Primary Cutaneous Melanoma

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Background: Focal adhesion kinase (FAK) is a cytoplasmic tyrosine kinase that mediates multiple functions such as cell survival, invasion and migration. FAK has been found to be over-expressed in multiple human cancers, including melanoma. We sought to examine the frequency and extent of FAK expression in a spectrum of melanocytic proliferations.

Design: Formalin-fixed, paraffin embedded sections from 40 melanocytic nevi (MN), 8 cases of melanoma in situ, 97 primary melanomas (PM) and 8 metastatic melanomas (MetM) were immunostained by automated methods (Ventana Medical Systems, Inc, Tucson, AZ) with a polyclonal antibody to FAK (BioSource, Camarillo, CA). A labeling index (LI) per 100 melanocytes was measured. Expression was correlated with histologic and prognostic variables.

Results: Cytoplasmic staining for FAK was found in 40% of MN (mean LI 40±22, range 10-80), 100% of MIS (68±36%, 10-100), 80% of PM (70±29, 10-100), and 100% of MetM (72±29, 20-100). The differences between MN and PM were significantly different, but not between PM and MetM. Of note, 14% of PM and 25% of MetM showed strong nuclear as well as cytoplasmic FAK labeling. FAK expression was most commonly found in large nests of MIS, at the invasive front of PM and MetM, and in microsatellites. No correlations were identified for FAK cytoplasmic expression with tumor thickness, Clark level, mitotic rate, tumor infiltrating lymphocytes, ulceration, microsatellites or disease free survival (DFS). However, FAK nuclear expression correlated with worse 5 year DFS (26% vs. 77%).

Conclusions: FAK cytoplasmic expression showed a significant increase in the histologic step from MN to PM and was most commonly found at the infiltrative margins of melanoma. This latter finding supports a role of FAK in tumor cell migration. Nuclear FAK expression correlated with poor DFS, implicating a role for aberrant cell-cycle signaling and aggressive disease.

413 Critical Domains of α6β4 Integrin for Squamous Cell Carcinoma Progression

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Background: Squamous cell carcinoma (SCC) is the second most common malignancy overall. Although metastasis is rare, local invasion and recurrency causes significant clinical problems. Recently, generation of malignant human epidermal tissue resembling SCC via overexpression of Ras/IκBα in transformed keratinocytes has been described. Formation of SCC-like neoplasms by transformed cells was found to be dependent on α6β4 integrin and laminin-5. The aim of this study was to identify extracellular as well as intracellular domains of α6β4 integrin critical for tumor formation, and investigate possible mechanisms by which this molecule exerts its effect on tumor progression.

Design: $\beta4^{NULL}$ human keratinocytes were retrovirally transduced to stably reexpress full length β4 integrin and β4 integrin mutant constructs. Keratinocytes were transformed with oncogenic Ras/ IκBα, and invasive potential as well as tumorigenic capacity *in vivo* were assessed. Phenotypic rescue experiments were carried out by constitutive overexpression of signaling intermediates which showed reduced activity levels in β4 integrin mutants.

Results: Invasive and tumorigenic capacity of Ras/IκBα transformed keratinocytes lacking endogenous expression of β4 integrin were significantly reduced, reexpression of wtβ4 integrin restored the invasive as well as proliferative potential. Transformed cells with specific cytoplasmic point mutations inhibiting binding of β4 integrin to plectin, as well as mutations in the extracellular domain disrupting ligand binding to laminin-5, both showed a phenotype comparable to $\beta4^{NULL}$ transformed keartinocytes. Constitutive activation of Rac1 lead to phenotypic rescue with restored invasive capacity as well as tumorigenic potential.

Conclusions: Apart from its function as an adhesive device in normal basal keratinocytes, β4 integrin is an important signaling intermediate required for Ras driven tumorigenesis in epidermal neoplastic tissue. Disruption of binding to the intracellular linker protein, plectin, as well as disruption of extracellular interaction with basement membrane components, impacts on the ability of transformed keratinocytes to proliferate and invade, two critical features for malignancy. Possible mechanisms of signal transduction include activation of small Rho GTPases. β4 integrin may therefore be a potential therapeutic target in regard to tumor formation and tumor survival signaling.

414 Genetically Heterogeneous and Clonally Unrelated Metastases May Arise in Patients with Cutaneous Melanoma

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Background: Melanoma of the skin frequently metastasizes to multiple regional lymph nodes and to distant sites. It is uncertain whether all metastases originate from the same tumor clone or whether the heterogeneity of the primary tumor is reflected in the multiple metastases.

Design: We examined 13 female patients who underwent biopsy or excision of a primary cutaneous melanoma and who subsequently underwent sentinel lymph node biopsy, regional lymph node dissection, and/or resection of distant metastases. All patients had multiple metastases (2 to 8) and a total of 13 primary tumors and 60 metastases were analyzed. Genomic DNA samples were prepared from formalin-fixed, paraffin-embedded tissue sections using laser-assisted microdissection. Loss of heterozygosity (LOH) assays for 5 microsatellite polymorphic markers on chromosome 1p36.3 (D1S214), 6q (D6S305), 9p21 (D9S171, *p16* gene), 11q23.3 (D11S528), and 9p (IFNA) were performed. In addition, X-chromosome inactivation analysis was performed on primary tumors and paired metastases from all patients.

Results: The overall frequency of allelic loss was 85% (11/13 patients) in the primary cutaneous melanomas and 92% (12/13 patients) in the metastatic tumors. In three cases the primary tumor and all metastases shared the same LOH pattern, consistent with a common clonal origin. Ten cases showed an LOH pattern in the metastases that differed from the pattern of allelic loss in the primary tumor. X-chromosome inactivation analysis showed the same pattern of non-random X-chromosome inactivation in both the primary melanoma and all of the lymph node and/or distant metastases in 7 of 11 informative cases studied. Four patients displayed an X-chromosome inactivation pattern in the primary tumor that differed from the corresponding metastases. In some cases, discordant, non-random X-chromosome inactivation was detected in multiple metastases of the same patient.

Conclusions: While the metastatic lesions and primary tumors appear to be clonally related in the majority of patients, we find evidence that clonally unrelated metastases may coexist in the same patient, a finding with interesting and important implications on the current concept of tumor biology and metastasis.

415 Loss of Heterozygosity Analysis Identifies Genetic Abnormalities in Mycosis Fungoides and Specific Loci Associated with Disease Progression

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Background: Mycosis fungoides (MF) exhibits a variety of underlying molecular defects. Loss of heterozygosity (LOH) is a technique used to detect chromosomal imbalances in neoplastic disorders. Examination of skin biopsies of MF in different stages and with different phenotypic changes for the presence of LOH at specific loci could lend further understanding to the underlying genetic aberrations involved in MF and its progression.

Design: We evaluated 19 patients with a total of 25 skin biopsies, including 15 plaque stage and 10 tumor stage specimens. We examined LOH at eight loci, D1S2766, IFNA, *p15* (D9S1748), *p16* (D9S171), *PTEN* (D10S2491, D10S541), D10S185 and *p53* (TP53), which are previously described sites of genetic abnormality in MF. The abnormal lymphocytes were microdissected from formalin-fixed, paraffin-embedded tissue sections, with adjacent uninvolved skin being used as an internal negative control. The LOH results of the MF cells were compared to adjacent normal skin, and at different sites and/or subsequent biopsies.
Results: Seventy-three percent of patients (14/19) showed genetic abnormalities, with sixteen of 25 (64%) lesions evaluated showing LOH in at least one locus. LOH was identified in 7 of 15 (47%) plaque stage lesions and in 9 of 10 (90%) tumor stage lesions. The three patients with sequential biopsies had increased allelic losses in tumor stage specimens compared with plaque stage. LOH involving Chromosome 10 was found in 7 of 10 (70%) tumor stage lesions. Loss of multiple alleles was only identified in tumor stage cases, with three tumor specimens undergoing allelic loss at 3 separate loci. Abnormalities were seen in 8% of lesions at 1p22, 9% of lesions at *p15*, 8% of lesions at 9p21 (IFNA), 4% of lesions at *p16*, 8% of lesions at D10S2491, 22% of lesions at D10S185, 42% of lesions at D10S541, and 8% of lesions at *p53*.
Conclusions: Our preliminary results suggest that LOH studies are a robust method for evaluating genetic abnormalities in MF. Tumor stage lesions manifest increasing allelic losses compared with plaque stage. Further, we found several loci on chromosome 10 that appear to be associated with progression from plaque to tumor stage MF.

416 Androgen Receptor and Cytokeratin 20 Immunohistochemical Stains Can Differentiate Morpheaform Basal Cell Carcinoma from Desmoplastic Trichoepithelioma

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Background: Patterns of androgen receptor (AR) and cytokeratin 20 (CK20) expression can distinguish conventional basal cell carcinoma from trichoepithelioma. Up to 78% of basal cell carcinomas express AR whereas trichoepitheliomas are typically negative for AR. The converse is found with CK20, where basal cell carcinomas retain no to rare Merkel cells and trichoepitheliomas contain normal to increased numbers of Merkel cells identified by CK20 expression. Within these two groups of tumors, morpheaform basal cell carcinoma (mBCC) and desmoplastic trichoepithelioma (DTE) are particularly challenging to differentiate. We investigated whether AR and CK20 immunostains can distinguish between mBCC and DTE.
Design: Immunohistochemistry for AR and CK20 was performed on 15 DTE and 20 mBCC. Any nuclear immunoreactivity within the tumor cells for AR or cytoplasmic outlining of cells within the tumor for CK20 was considered positive. Differences in expression of AR and CK 20 were analyzed by the Chi square test.
Results: The AR+CK20- immunophenotype was present in 9/20 (45%) mBCC, but none of the DTE (p-value=.0237). The AR-CK20+ immunophenotype was present in 13/15 (87%) DTE, but none of the mBCC (p-value=<.0001). An inconclusive AR+CK20+ immunophenotype was present in 2/15 (13%) DTE and 1/20 (5%) mBCC.
Conclusions: Immunohistochemical stains for AR and CK20 are useful in differentiating DTE from mBCC. The AR-CK20+ immunphenotype is sensitive (87%) and specific for DTE (100%). The AR+CK20- immunophenotype is specific for mBCC (100%) but not very sensitive (45%). Rare cases of both DTE and mBCC are AR+CK20+, and this pattern of expression should be considered inconclusive.

417 Melanocytic "Ball-in-Mitts" and "Microalveolar Structures" and Their Role in the Development of Cellular Blue Nevi

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Background: It is unknown how cellular blue nevi (CBN) come into being. To answer the question, one obviously must identify the smallest lesion with the microscopic features of a pure CBN. The literature contains no illustrated case of a small (<5mm)pure CBN; they are always combined nevi (CoN).
Design: To test the hypothesis whether CBN may originate from "ordinary" "non-blue" compound and dermal nevi, a total of 275 melanocytic nevi including 59 CBN, 34 ordinary blue nevi (OBN), 87 CoN (including 43 so-called clonal nevi), 35 deep penetrating nevi (DPN), and 60 "ordinary" compound and dermal nevi (30 of each) were studied for the presence of so-called ball-in-mitts (BiM) and microalveolar structures (MaS).The former were defined as a single centrally placed melanocyte with a round to oval nucleus (the "ball" cell) and a clear, dusty or pigmented cytoplasm encircled by a single dendritic cell (the "mitt" cell) with an oval to spindle-shaped nucleus and slender bipolar processes containing melanin and surrounding at least one-forth of the ball's diameter. A MaS was defined as a group of 2-10 centrally placed melanocytes with round to oval nuclei and clear, dusty or pigmented cytoplasm (balls) surrounded by one or more cells (mitts) with spindle-shaped nuclei and slender bipolar processes containing melanin.
Results: Microscopically, BiM and MaS were detected in all types of nevi studied, with the highest incidence in CoN (82%), CBN (76%) and "ordinary" "non-blue" nevi (73%). In CBN, BiM and MaS tended to be located in the deeper portion of the lesions, while in "ordinary" "non-blue" nevi they were most often found superficially, and in clonal nevi, these structures were often confined to the "clonal" parts. Immunohistochemically, BiM and MaS were positive for HMB45. Ultrastructurally, the balls had round to oval nuclei, while the mitts possessed oval, elongated to spindled nuclei. Melanosomes were found in various stages in the cells of both structures. The cytoplasm of the mitts typically formed elongated polar processes, sometimes with club-like widenings at the ends, completely or partially encircling the balls. In the MaS, the adjacent cells forming the mitts surrounded the ball cells like a chain.
Conclusions: Some or even most cases of CBN may evolve from "ordinary" "non-blue" nevi. This process may involve several steps and is probably reflected by the appearances of CoN, DPN, CBN that often show a morphologic overlap, and BiM and MaS found in various stages of their development seem to greatly account for this overlap.

418 Identification of Genomic Predictors of Non-Melanoma Skin Cancer in Solid Organ Transplant Recipients

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Background: With advances in transplant medicine, the long-term survival of solid-organ transplant recipients (SOTR) is increasing. The drawback to this improved survival is a corresponding increase in the rates of complications such as post-transplant malignancy. The most common tumors to occur in these patients are non-melanoma skin cancers (NMSC), which include basal and squamous cell carcinoma (BCC and SCC), and have a more aggressive clinical course than those seen in non-transplant patients, with increased risks of recurrence, metastases and death. Immunosuppressive drugs appear to contribute to the development of tumors, however genetics also play a role since not all SOTRs will go on to develop NMSC. Although several studies have examined mechanisms underlying the formation of NMSC, no studies have yet focused on the molecular genetics of cancers in SOTRs.
Design: With informed consent, skin tumors were obtained at the time of resection from the University Health Network Division of Dermatology, Toronto, Canada. BCCs and SCCs from 6 SOTRs and 6 non-transplant patients will be compared using the Human Genome U133A 2.0 plus GeneChip® arrays (Affymetrix, Santa Clara, USA), which contain 14,500 unique genes. Differentially expressed genes between tumors from non-transplanted compared to transplanted patients will be identified and validated using quantitative real-time PCR (QRT-PCR) and immunohistochemistry (IHC) in a larger patient cohort.
Results: Tissue was snap-frozen and stored at -80C until RNA extraction and microarray analysis. Thus far, a subset of patient samples has been analyzed using unsupervised hierarchical clustering. Data analysis shows distinct clustering of tumors from transplanted and non-transplanted patients. Preliminary data using Significance Analysis of Microarrays (SAM) identified 123 under- and 29 over-expressed genes between the distinct sample clusters. These genes are currently being validated using QRT-PCR and IHC, and include genes involved in apoptosis (*APP*), cell adhesion (*MMP1, TNC*) and cell cycle regulation (*JUN*).
Conclusions: Global gene expression analysis is able to identify differentially expressed genes in tumors from transplant compared to non-transplant patients. Validated genes will lead to a better understanding of the mechanism underlying the formation of NMSC and will identify biomarkers for diagnosis and gene targets for therapy.

419 Loss of Expression of a Carney Complex Gene, Protein Kinase A Regulatory Subunit 1α, in Sporadic and Carney Complex-Associated Pigmented Epithelioid Melanocytoma

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Background: Pigmented epithelioid melanocytoma (PEM) is a recently described entity comprising most cases previously considered to be so-called "animal-type melanoma", and histologically indistinguishable from epithelioid blue nevus (EBN) occurring in patients with Carney complex (CNC). Protein kinase A regulatory subunit type 1α (PRKAR1α or R1α) is a tumor suppressor gene located on chromosome 17 that is mutated in more than half of CNC families; loss of heterozygosity can be demonstrated in EBN lesions in these CNC patients. In this study, we examined whether PEM and EBN are related on a molecular level and whether changes in expression of the CNC gene R1α may be involved in the pathogenesis of PEM.
Design: Histological analysis of H&E stained sections and immunohistochemistry (IHC) with R1α antibody were performed on 37 sporadic PEMs, 9 Carney-complex associated PEMs, 5 equine dermal melanomas, and 236 benign and malignant melanocytic tumors (66 conventional sections consisting of 9 compound nevi, 10 Spitz nevi, 5 deep-penetrating nevi, 5 blue nevi, 6 cellular blue nevi, 2 malignant blue nevi, 3 lentigo maligna, and 26 melanomas of various types; and 170 tissue microarray sections consisting of 35 benign nevi, 60 primary melanomas and 75 metastastic melanomas). Histologic diagnoses were based on preexisting pathologic reports and were confirmed for this study. Two pathologists confirmed IHC for R1α.
Results: IHC showed that R1α was expressed in all but one core from tissue microarrays (169/170), and in all 66 melanocytic lesions evaluated in conventional sections. In contrast, R1α was not expressed in the 9 EBN from patients with CNC. Expression of R1α was lost in 27 of 37 PEMs, but expressed in 5 equine melanomas studied.
Conclusions: The results support the concept that PEM is a distinct melanocytic tumor occurring in a sporadic setting and in the context of CNC. They also suggest that PEM differs from melanomas in equine melanotic disease, further arguing that the term animal-type melanoma may be a misnomer for this group of lesions. Loss of expression of PRKAR1α offers a useful diagnostic test that helps to distinguish PEM from its histological mimics.

420 Gene Expression Microarray System Used as a Molecular Diagnostic Tool To Distinguish Malignant Melanoma vs Benign Nevi from FFPE Tissue

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Background: Distinction of melanoma from a benign nevus may be very difficult when only standard histologic criteria are utilized. A study of 11 expert pathologists reviewing 37 classic melanocytic lesions showed that they were in agreement in only 30% of the cases (Hum. Pathol. 1996;27:1115-1116). Since the clinical management and prognosis of patients is entirely dependent on pathologic diagnostic accuracy, the goal of our study is to develop an objective molecular diagnostic method using the gene expression microarray system to distinguish melanoma from nevi of all types.

Design: 38 melanomas and 48 common nevi were retrieved from the archival files. Slides were created from formalin fixed paraffin embedded (FFPE) blocks and cells of interest were isolated with laser capture microdissection. Total mRNA was isolated, amplified using a modified T7-T3 amplification protocol (Genisphere), and labeled. The samples were then hybridized to a 12K CustomArray (Combinatrix, Seattle, WA, USA) using a two color format and a Stratagene universal RNA reference. Data analysis was performed using GeneMaths to create a gene list database.
Results: There was a significant difference in gene expression of the most altered genes distinguishing melanoma from nevi. An initial 50 gene signature was created and found to be consistent with some previously identified genes that had strong correlation with melanoma and melanocytic lesions. Our method showed melanomas, relative to nevus, had increased expression of some genes such as PCNA, GSPT1, PHACTR1, Stat1, and ARPC2 (p=1.19E-05 to 5.43E-07) and decreased expression of FABP7, DLC1, GPX3, and Ellsl (p=8.06E-06 to 1.30E-09), to name a few within our 50 initial gene signature.
Conclusions: The distinctive gene expression profile of melanoma and nevi offers the ability to distinguish them by an objective molecular measure. This offers the possibility of utilizing the gene expression microarray as a future molecular diagnostic tool to distinguish melanoma from indeterminate melanocytic lesions with high sensitivity and specificity. Additionally, the use of FFPE tissues increases practicality since the majority of clinical specimens are formalin fixed/paraffin embedded, facilitating the movement of microarray based technologies from the lab bench to the clinical bedside.

421 The Utility of Apo D as a Diagnostic Immunohistochemical Adjunct in Dermatofibrosarcoma Protuberans

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Background: Although the histology of dermatofibrosarcoma protuberans (DFSP), a slow-growing locally aggressive tumor of disputed histogenesis, is fairly distinctive, immunohistochemical confirmation is a required ancillary tool. The most used marker to date is CD34, the human progenitor cell antigen. However, CD34 is negative in 10-20% cases of DFSP. A recent study indicates that Apo D, a 33-kD glycoprotein component of high-density lipoprotein, is highly expressed in DFSP. The goal of this study was to assess the expression of Apo D in DFSP in an effort to ascertain its specificity and utility as an immunohistochemical adjunct.
Design: Immunohistochemical staining was performed using Apo D (clone 36C6, 1: 80 dilution, Novocastra, Newcastle, UK) and CD34 (1:160 dilution, Dako) on 25 cases of DFSP and 6 cases of dermatofibroma (DF, including 4 cellular). Staining was categorized as being diffusely positive, focally positive or negative.
Results:

Table. Immunohistochemical staining of DFSP and DF for Apo D and CD34.

		Apo D			CD34	
	Diffuse positive	Focal positive	Negative	Diffuse positive	Focal positive	Negative
DFSP	19 (76%)	5 (20%)	1 (4%)	21 (84%)	1 (4%)	3 (12%)**
DF	0	4 (67%)*	2 (33%)	0	2 (33%)*	4 (67%)

*In one of these cases positivity for both Apo D and CD34 was evident only at the periphery of DF and in one case only in the lesional area extending into the subcutis.**All three were positive (1diffusely, 2 focally) for Apo D.

Conclusions: In DFSPs, the expression of Apo D parallels that of CD34 and appears to show similar specificity based on the absence of diffuse positive staining in all cases of DF. The diagnostic utility of Apo D is highlighted by its expression in cases of CD34-negative DFSPs.

422 Genomic Gains of *COL1A1-PDGFB* Occur in the Evolution of Giant Cell Fibroblastoma into Dermatofibrosarcoma Protuberans: A Study of 13 Cases with Morphologic Correlations

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Background: Giant cell fibroblastoma (GCF) is a subcutaneous mesenchymal neoplasm characterized by the chromosomal t(17;22). This translocation leads to the formation of the fusion gene *COL1A1-PDGFB*, which is also seen in the supernumerary ring chromosome of dermatofibrosarcoma protuberans (DFSP). Several studies have addressed the molecular genetics of DFSP but molecular cytogenetics analyses with morphologic correlations have not been done in GCF and GCF/DFSP hybrids. Herein we studied the genomic structure of *COL1A1-PDGFB* in GCF and GCF/DFSP hybrids, and identified the molecular cytogenetic signatures in individual cells in both GCF and DFSP components.
Design: Four pure GCF and 9 GCF/DFSP hybrids (DFSP areas representing 5 to 70% of the tumor area) were studied. All tumors exhibited classical histological features and expressed CD34. *COL1A1* and *PDGFB* rearrangements were evaluated by fluorescence in situ hybridization (FISH) using custom designed probes for *COL1A1* and *PDGFB* on paraffin-embedded thin tissue sections. Approximately 400 cells were analyzed in each tumor component by two independent investigators.
Results: All GCF and GCF/DFSP hybrids showed unbalanced rearrangements of *COL1A1-PDGFB*. Seven (4 pure GCF and 3 GCF/DFSP hybrids) showed a single copy of *COL1A1-PDGFB*. Six GCF/DFSP hybrids showed copy gains of *COL1A-PDGFB* in both GCF and DFSP components but these predominantly occurred in the DFSP component. None of the pure GCFs showed additional copies of *COL1A-PDGFB*. The molecular cytogenetic abnormalities were found not only in spindle/stellated cells of both GCF and DFSP components but also in individual nuclei of the multinucleated giant cells.
Conclusions: Both pure GCF and GCF/DFSP hybrids showed unbalanced rearrangements of *COL1A-PDGFB* at the molecular cytogenetic level. Genomic gains of *COL1A-PDGFB* were found predominantly in the DFSP component of GCF/DFSP hybrids but in none of the pure GCF, suggesting that gains of *COL1A-PDGFB* are associated with the progression of GCF into DFSP. These molecular cytogenetic abnormalities were found not only in the spindle/stellated cells but also in individual nuclei of the multinucleated giant cells, suggesting that these cells may result from the fusion of individual neoplastic cells.

423 Alpha-Methylacyl Coenzyme A Racemase Is Immunoreactive in Extramammary Paget's Disease

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Background: Immunostaining for alpha-methylacyl coenzyme A racemase (AMACR) has become a common procedure in the diagnosis of morphologically difficult prostate carcinoma. Initially considered a specific marker of prostatic carcinoma, AMACR has been found in a variety of other neoplasms. In this study, we report findings in 21 cases of extramammary Paget's disease (EMPD) stained for AMACR, a neoplasm not previously reported to show reactivity for this marker.
Design: The index case was an 87 year old man with a history of prostate carcinoma, who presented with phimosis and balanoposthitis. The circumscision specimen showed Paget's disease, with expression of AMACR, raising the question of whether expression of this marker is characteristic of EMPD generally, or is found only in EMPD associated with prostate carcinoma. To answer this question, 20 additional EMPD were stained for AMACR (P504S) and for CK7. The additional patients included 10 men and 10 women, with sites of involvement among the men including scrotum (n = 6), groin (n = 5), buttock (n = 2), thigh (n = 1), penis (n = 1), and perianal skin (n = 1). Involvement in women was limited to the vulva. Only one of these patients had an associated malignancy, a man with colorectal carcinoma diagnosed concurrently with perianal EMPD. Staining for AMACR was also performed in six patients with pagetoid Bowen's disease (PBD) and in eight patients with mammary Paget's disease (MPD). Staining was graded in intensity (negative, 1+, 2+, 3+), and distribution (focal or diffuse).
Results: Immunoreactivity for AMACR was seen in 71% of EMPD overall (15/21), in 82% of men (9/11), and in 60% of women (6/10). Among men, staining was 2+ in 45% (5/11), 1+ in 36% (4/11), diffuse in 36% (4/11), and focal in 45% (5/11). Among women, staining was 2+ in 40% (4/10), 1+ in 20% (2/10), diffuse in 50% (5/10), and focal in 10% (1/10). Expression of CK7 was seen in 86% of cases overall (18/21), with diffuse, 3+ staining in all positive cases. Expression of AMACR was seen in six of eight patients with MPD (75%), and in one of six patients with PBD (17%).
Conclusions: Immunoreactivity for AMACR is common in EMPD, a previously unreported finding. It is found in EMPD in both men and women, and is not limited to patients with associated malignancies such as prostate carcinoma, which also express this marker.

424 Cutaneous Extra-Ocular Sebaceous Carcinoma: A Less Aggressive Neoplasm Than Previously Asserted

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Background: Extra-ocular sebaceous carcinoma is a rare entity said to be locally aggressive with capability for metastatic spread, typically found on the head and neck. Some cases have arisen in areas of prior radiation exposure; others are seen in Muir-Torre Syndrome. The literature reveals little about prognostic features, with the largest reported case series encompassing a total of seven cases.
Design: We present 21 cases of extra-ocular sebaceous carcinoma in 19 separate patients. Histological characteristics of the lesions were reviewed, including: location, size, depth, level of invasion from the skin surface, pattern, grade, mitotic activity, ulceration, comedo necrosis, pagetoid spread, lymphovascular and perineural invasion. Comprehensive chart review was performed on all available cases, with data retrieved on a total of 13 separate patients.
Results: 13 of 22 cases (62%) were found on the head and neck; 5 of these were located in or around the nose. Tumor size ranged from 0.05 cm to a maximum of 3.0 cm, with an average of 1.0 cm. The tumors averaged 0.71 cm in thickness from the overlying skin surface, ranging from 0.05 to 2.2 cm. Most cases were found in the deep reticular dermis or subcutis corresponding to a Clark level of IV (8/18) or V (8/18). Four lesions showed ulceration and 6 showed comedo necrosis. No lymphovascular invasion was noted. Perineural invasion was seen in only one lesion. Mitotic activity varied from 0 to 21 mitoses per ten high power field (hpf), with an average of 8 per 10 hpf. Six tumors showed a pushing growth pattern; 13 showed an infiltrative pattern. Seven lesions showed pagetoid spread. Seven of the 13 patients for which clinical data were available had Muir-Torre syndrome. Two had colon cancer; 1 had small intestinal adenocarcinoma; 4 had breast cancer; and 1 endometrial cancer. Only one patient had been treated with radiation to the lesional area (face), in this case for acne as a child. None of the 13 cases had a local recurrence; one of 13 had a metastasis to the same arm as his original lesion. All of the patients for which greater than 12 months of follow up were available were alive at the time of follow-up. Follow-up ranged from 0 to 95 months. The lesion which metastasized was large (2.7 cm), but no lymphovascular invasion was noted.
Conclusions: The vast majority of extra-ocular sebaceous carcinomas, especially small lesions, are associated with a favorable clinical course akin to basal cell carcinoma. Metastasizing extra-ocular sebaceous carcinomas are very rare.

425 Myxoid Dermatofibrosarcoma Protuberans: Clinicopathologic, Immunohistochemical, and Molecular Analysis of 6 Cases

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Background: Dermatofibrosarcoma protuberans (DFSP) represents a locally aggressive mesenchymal neoplasm of skin and subcutis with characteristic clinicopathologic, immunohistochemical and molecular findings. In addition to typical cases morphologic variants as pigmented, fibrosarcomatous, myofibroblastic, and granular cell DFSP have been described. Purely or predominantly myxoid DFSP is extremely rare, and may cause considerable diagnostic problems. Six cases of predominantly myxoid DFSP were studied.

Design: Paraffin embedded blocks and slides were retrieved from the files of the authors. Clinical data were obtained from the referring pathologists and dermatologists. Immunhistochemistry was performed using the ABC-method, and selected cases were studied by PCR-technique.

Results: There were 4 male and 2 female patients (age ranged from 29 to 74 years). Locations included the inguinal area (3 cases), upper arm, shoulder, and back (one each). The patients were treated by local or wide excision. Tumor size ranged from 1.5 to 12 cm. Histologically, a nodular growth with peripheral diffuse infiltration as well as a diffusely infiltrating growth of relatively uniform spindled and stellated tumor cells was noted. Three cases were entirely myxoid and in 3 cases more than 80% of the tumor area showed myxoid changes. At least focally, hypocellular areas were evident in 2 cases. In 1 case each focal fibrosarcomatous and giant cell fibroblastoma-like changes were present. Scattered enlarged tumor cells were seen in 1 case. The mitotic rate ranged from 2 to 20 mitoses in 10 high power fields. Numerous blood vessels with slightly fibrosed vessels were seen in 5 cases. Immunohistochemically, tumor cells in all cases stained positively for CD34, and in 1 case a focal expression of actin was noted. The remaining antibodies (CD99, CD31, S-100, EMA) were all negative. PCR-technique showed in 1 case the characteristic *COL1A1-PDGFB* fusion gene, while 1 case proved negative. Follow-up informations in 3 cases revealed a local recurrence at 5 years.

Conclusions: Myxoid DFSP represents a very rare morphologic variant that has to be distinguished from benign and malignant myxoid mesenchymal neoplasms as superficial acral fibromyxoma, myxoid solitary fibrous tumor, myxoid perineurioma, low-grade fibromyxoid sarcoma, myxoid liposarcoma and myxoid synovial sarcoma.

426 p16 Is a Relevant Predictor of the Lymph Node Status by Melanoma Patients

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Background: Up to date the only therapy to cure melanoma patients is surgical excision of localized, non-metastatic primary cutaneous melanoma (stage I and stage II). Unfortunately, even 20% of those patients have already micrometastatic disease. Currently, there is no cure for patients who present with metastasis. Therefore, identifying patients at increased risk for metastasis is one of the most critical issues in the management of melanoma. These patients have to be considered for adjuvant therapy. Loss of the tumor suppressor gene *p16* has been shown to be associated with tumor progression of melanoma. In this study the correlation of p16 protein expression in the primary tumor with lymph node status and tumor-specific survival was investigated.

Design: We investigated 64 primary cutaneous melanomas for the presence of the product of the tumor suppressor gene *p16*, using immunohistochemistry. All patients had a sentinel biopsy at time of primary diagnosis. Thirty-four skin nevi were used as control. We observed three different staining patterns: Nuclear and cytoplasmic staining, exclusive cytoplasmic staining and absence of p16.

Results: There was a significant difference in p16 expression between nevi and melanoma. Fourteen of 64 (22%) melanomas were negative for p16, whereas all 34 nevi displayed a nuclear and cytoplasmic p16 staining. The level of p16 expression decreased from benign nevi to melanoma without metastasis to melanoma with metastasis. Exclusive cytoplasmic immune staining correlated significantly with absence of lymph node metastasis ($p<0.05$). Death of disease correlated with absence of p16 immunostaining ($p=0.01$).

Conclusions: The presence of p16 protein expression in primary malignant melanoma is associated with negative lymph node status and increased tumor specific survival. These results indicate the relevance of p16 expression as a prognostic marker in melanoma patients.

427 The Utility of PU.1 as an Immunohistochemical Marker for Histiocyte Lesions of the Skin

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Background: General surgical pathologists are familiar with the use of CD68, a histiocyte/macrophage marker, which has been advanced as a histiocytic marker. Although CD68 is currently the most widely used marker for histiocytic lesions, it is not specific and the stain itself may be difficult to interpret due to variability in cytoplasmic staining. A more specific histiocyte immunohistochemical marker has yet to be unveiled. PU.1 is a transcription factor restricted to the hematopoietic system. It is expressed in myeloid lineage and B-lymphocytes, but is absent in T- and non-hematopoietic cells. Among myeloid lineage derived cells PU.1 is over-expressed in monocytes, histiocytes, and dendritic cells. Currently there have been no studies of PU.1 expression in primary cutaneous histiocytic and dendritic lesions of the skin.

Design: Immunohistochemical staining was performed on formalin fixed paraffin embedded sections of cutaneous lesions using an antibody to the PU.1 gene product. Sixty-two total cases were stained, including 9 reticulohistiocytomas, 9 Langerhans cell histiocytoses, 7 juvenile xanthogranulomas, 9 fibrous papules, 8 dermatofibromas, 12 dermatofibrosarcoma protuberans (DFSP), 4 Spitz nevi, and 4 malignant melanomas.

Results: Strong nuclear staining for PU.1 was seen in all the cases of histiocyte origin including 9/9 reticulohistiocytomas, 9/9 Langerhans cell histiocytoses, and 7/7 juvenile xanthogranulomas. No staining for PU.1 was seen in all studied cases of fibrous papules, the DFSPs, dermatofibromas, Spitz nevi, and malignant melanomas.

Conclusions: PU.1 is a highly specific and sensitive immunohistochemical marker for identifying cutaneous histiocyte-derived lesions. PU.1 staining is easily interpreted due to the sharp nuclear staining as compared to the irregular and often variable cytoplasmic staining seen with CD68.

428 Comparison of PHH3, Ki-67 and Survivin Immunoreactivity in Benign and Malignant Melanocytic Lesions

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Background: Differentiating malignant melanoma (MM) from benign melanocytic lesions can be challenging. This study evaluated the use of the immunohistochemical mitosis marker phospho-Histone H3 (pHH3), and the proliferation markers Ki-67 and survivin in separating MM from benign nevi.

Design: Formalin-fixed, paraffin-embedded sections from 38 melanocytic lesions (10 malignant melanomas, 8 Spitz nevi, 10 dysplastic nevi and 10 compound nevi) were stained with antibodies to pHH3, Ki-67 and survivin. For pHH3, the total number of mitoses in dermal melanocytes per 10 high power fields (HPF) was determined. For Ki-67 and survivin, one thousand cells were counted randomly in the dermal component of each tumor, and the labeling index was recorded as the percentage of positive nuclei.

Results: No pHH3 expression was detected in the dermis of compound and dysplastic nevi. Rare mitoses were observed in the superficial dermis in 3/8 Spitz nevi (37%). One Spitz nevus showed 2 mitoses and 2 showed 1 mitosis each. Staining for pHH3 was higher in MM (average: 23/10HPF; range: 2-75/10HPF) than in Spitz nevi (average: 0.5/10HPF; range: 0-2/10HPF), and was evenly distributed throughout the whole MM lesion compared to a superficial dermal location in Spitz nevi. There was no cytoplasmic staining for survivin in any of the 38 melanocytic lesions and no nuclear staining in any of the benign ones. Survivin nuclear staining was present in all 10 cases of malignant melanoma with an average index of 6% (range: 1-13%). In benign melanocytic lesions, the Ki-67 index was less than 4% (average: 1-2%; range: 1-4%) and staining was present predominantly close to the dermo-epidermal junction, compared to an index of at least 5% in melanomas (average: 21%; range: 5-50%) and a generally dispersed pattern of staining throughout the whole lesion.

Conclusions: PHH3, Ki-67, and survivin can be useful adjuncts to histopathology to separate MM from benign melanocytic lesions. PHH3 is especially useful to highlight mitoses, and to rapidly assess the mitotic activity in melanocytic lesions.

429 *FUS* Flourescence *In-Situ* Hybridization Is Useful for Differentiating Cutaneous Low-Grade Fibromyxoid Sarcoma from Other Superficial Fibromyxoid Neoplasms

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Background: Low-grade fibromyoxid sarcomas (LGMFS) are rare, typically deep soft tissue neoplasms which have deceptively bland cytology and the potential to metastasize. A t(7;16)(q34;p11) *FUS-CREB3L2* has been identified in approximately 80-90% of deep soft tissue LGFMS. Cutaneous fibromyxoid neoplasms are not uncommon, but dermatopathologists rarely consider LGFMS since this lesion is so uncommon in the skin. We identified a subset of superficial LGFMS and a spectrum of neoplasms within the differential and performed fluorescence *in-situ* hybridization (FISH) to evaluate the utility of *FUS* rearrangement in the workup of cutaneous fibromyxoid neoplasms.

Design: FISH for chromosomal rearrangement of *FUS* (16p11) (Abbott Molecular/Vysis, Des Plaines,IL) using a dual color break-apart format probe was performed on formalin-fixed, paraffin-embedded tissue (FFPET) sections from superficial LGFMS (n=6), myxomas (n=10), and myxofibrosarcoma/myxoid malignant fibrous histiocytomas (myxoid MFH) (n=5). 100 non-overlapping tumor nuclei per case were evaluated for evidence of either fused (normal) or split (translocated) signals.

Results: Of the LGFMS, 4/6 (67%) showed a rearrangement of *FUS* (range: 72-80% positive nuclei per 100 nuclei). One of the two *FUS*-negative cases was composed predominantly of areas resembling hyalinizing spindle cell tumor with giant rosettes. The other neoplasms within the differential were devoid of any rearrangement involving *FUS* (range: 0-2% positive nuclei per 100 nuclei).

Conclusions: Our observed frequency of *FUS* rearrangement in superficial LGFMS is consistent with those published in the literature for more deeply seated lesions. When applied to suspicious superficial myxoid or fibromyxoid neoplasms, the *FUS* FISH probe in FFPET can be a useful ancillary technique for diagnosis of this uncommon and deceptively bland tumor.

430 Role of the Bulge Region in Scarring Alopecia

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Background: While the pathogenesis of most scarring alopecias is poorly understood, one recent study indicates destruction of follicular stem cells, residing in the bulge region, as a possible mechanism in LPP, the prototypic scarring alopecia. Using a panel of antibodies including one specific for the bulge region, the aim of our study was to ascertain the target of inflammation and, to more precisely characterize the inflammatory infiltrate in various scarring alopecias.

Design: Sixteen cases of scarring alopecias including LPP (8), pseudopelade of Brocq (2), traction alopecia (TA, 2), discoid lupus erythematosus (DLE, 1), follicular degeneration syndrome (FDS, 1), folliculitis decalvans (FD, 1) and chronic infectious folliculitis (1) were selected for the study. Immunohistochemical studies were performed using a panel of antibodies that included anti-cytokeratin (CK) 15, specifically targeting follicular stem cells and, CD4, CD8, CD1a, and HLA- DR to characterize the inflammatory infiltrate. Results were analyzed using semiquantitative criteria (table 1).

Results: Table 1.

	CD4	CD8	CD1a	HLA-DR	Diagnosis
Case 1*	+++	+	++	+++	LPP
Case 2*	+++	+	-	++	Chronic Folliculitis
Case 3*	++	-	-	+	LPP
Case 4*	+++	+	-	+++	LPP
Case 5*	+++	+	-	+++	TA
Case 6*	+++	-	-	++	LPP
Case 7*	+++	++	-	+++	DLE
Case 8	+	-	-	+++	LPP
Case 9*	++	-	-	++	TA
Case 10**	+	-	-	++	Pseudopelade vs TA
Case 11**	+	-	-	+	FDS
Case 12*	++	-	-	+++	LPP
Case 13*	++	+	-	++	LPP
Case 14**	-	-	-	-	Pseudopelade vs TA
Case 15*	+++	+	+	+++	FD
Case 16*	+++	++	+	+++	LPP

+ = scant to few cells; ++ = moderate; +++ = numerous

While the bulge region was identified in all 16 cases, CK15 staining was absent in inflamed hair follicles(*)(75%) and present in the hair follicles surrounded by fibrosis (**)(19%). Twelve of sixteen cases showed a moderate to dense perifolliclular inflammatory infiltrate of predominantly CD4+, HLA-DR+ lymphocytes.

Conclusions: Our data indicate that the bulge region is not necessarily involved in all of the scarring alopecias. While the absence of CK15 staining may be a consequence of inflammatory destruction of follicular stem cells, the paucity of CD8+ T cells in the inflammatory infiltrate, evident in all of our cases, indicates lack of a cell-mediated cytotoxic immune response in scarring alopecias and argues against this as a possibility.

431 K Homology Domain Containing Protein Overexpressed in Cancer (KOC) Is Expressed in Malignant Melanoma but Not in Melanocytic Nevi

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Background: K homology domain containing protein overexpressed in cancer (KOC) is a member of the insulin-like growth factor (IGF) mRNA-binding protein (IMP) family and is expressed during embryonic development and in certain malignancies. KOC, also known as L523S and IMP3, acts to promote tumor cell proliferation by enhancing IGF-II protein expression. KOC expression in malignant melanomas and melanocytic nevi has not been investigated.

Design: Thirty-three surgically excised or biopsied melanocytic lesions, including 14 malignant melanomas (13 skin with Breslow depth from 0.275 mm to 1.3 cm and one metastasis in soft tissue of the arm), 8 dysplastic nevi, and 11 benign nevi were immunohistochemically studied using a monoclonal antibody against KOC/L523S, clone 69.1. A lesion was recorded positive if more than 10% of the lesional cells showed cytoplasmic staining. The immunostaining intensity was graded as weak, moderate, or strong. P value of <0.05, as determined by Fisher exact test, was considered statistically significant.

Results: Seven of 14 (50%) malignant melanomas, including one metastatic melanoma in soft tissue of the arm, showed moderate to strong positive staining for KOC, with 4 cases exhibiting positivity in >90% of tumor cells and 3 cases showing positivity in 10-20% of tumor cells. All four (100%) cases with Breslow depth of >1.3 mm showed moderate to strong positive staining for KOC while 2/9 (22%) cases with Breslow depth ≤1.3 mm exhibited moderate to strong positive staining for KOC, and they are significantly different ($p<0.05$). No positive KOC staining was detected in 8 dysplastic nevi and 11 benign nevi.

Conclusions: 1. KOC is expressed in malignant melanoma but not in melanocytic nevi even when dysplastic features are present. 2. KOC positivity parallels Breslow depth of invasion. 3. These findings indicate that KOC is a marker of aggressiveness in the malignant melanoma; and KOC may play an important role in the regulation of biological behavior of this tumor.

432 Myxoid Dermatofibrosarcoma Protuberans: A Rare Variant Analyzed in a Series of 23 Cases

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Background: Dermatofibrosarcoma protuberans (DFSP) is a slowly growing locally aggressive tumor, with a high recurrence rate and a very low metastatic potential. Although it is not uncommon for DFSP to have focally myxoid areas, extensively myxoid DFSPs are rare and frequently present a diagnostic challenge.

Design: 23 myxoid DFSPs (defined as DFSP greater than 50% myxoid) were retrieved from the authors' consult files. Cases were analyzed for growth pattern, borders, cytology, stroma, and mitotic rate. Immunohistochemistry for CD34, S100, SMA, and HHF-35 was performed. Clinical data were obtained from the referring pathologists.

Results: 13 patients were male and 10 were female (median age 40 years; range 9 months to 72 years). Submitting pathologists' diagnosis included liposarcoma, superficial angiomyxoma, low grade fibromyxoid sarcoma, myxofibrosarcoma, neurofibroma, nodular fasciitis, DFSP, and myxoma. Tumor size ranged from 1.5 to 11 cm (mean 3.4 cm). The most frequent sites were the extremities (9) and head and neck (7), followed by the trunk (4) and pelvic region (3). Tumors ranged from 50 to 100% myxoid (mean 76.3 %). The majority of cases displayed an infiltrative sheet-like proliferation of uniform spindle cells with pale to eosinophilic cytoplasm and stellate nuclei, with usually only very focal distinctive storiform areas. All cases displayed honeycomb infiltration of fat. The stroma was myxoid with prominent thin-walled vessels. Mitoses ranged from 0 to 5 per 10 hpf. 95 % of cases were CD34 positive and all cases were negative for S100 and muscle markers. Clinical follow-up, ranging from 3- 21 years, (mean follow-up 8.3 years), revealed local recurrence in 2 cases and no evidence of metastasis. All patients were disease free following either wide excision or excision followed by radiotherapy.

Conclusions: Myxoid DFSP is a distinct variant of DFSP and appears to have a similar prognosis and clinical presentation to more typical DFSP. Due to its unusual appearance, myxoid DFSP presents a difficult histologic diagnostic challenge and seems often to go unrecognized. The proper identification and diagnosis of myxoid DFSP requires recognition of the stromal and cytologic features, immunohistochemical pattern, and adequate sampling, to allow for the identification of more typical areas of DFSP. Recognition of this DFSP variant is important since the histologic differential includes both benign and malignant tumors, which could lead to under or over treatment respectively.

433 Overexpression of FGF-2 May Confer a Survival Advantage to Tumor Cells in Melanoma and Dysplastic Nevus with Regression

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Background: Regression is a host immune response against melanocytes mediated by lymphocytes, sometimes observed in melanoma (M) and dysplastic nevus (DN). Melanoma and nevus cells are usually resistant to the apoptotic effects of transforming growth factor β (TGFβ), partly by activation of fibroblast growth factor-2 (FGF-2) since this latter restores Bcl-2 levels in melanoma cell lines.

Design: We determined expression of TGFβ and FGF-2 by immunohistochemistry in 25 cases each of M and DN showing features of regression. Lesions were scored as follows: 0 for less than 5% of cells expressing the marker, 1 for 5-25%, 2 for 26-75%, and 3 for over 75%. Intensity was evaluated as 0, 1, 2, and 3.

Results: Expression of TGFβ was higher in the DN than in the M group ($p<0.001$), whereas there was no statistical difference in the expression of FGF-2 between M and DN. In both groups of lesions, the percentage of melanocytes expressing FGF-2 was higher than those expressing TGFβ ($p<0.001$). Furthermore, in the DN group, the intensity of FGF-2 expression by melanocytes was higher than that of TGFβ ($p<0.001$).

Conclusions: Our results suggest that *in vivo* overexpression of FGF-2 may be an adaptive mechanism in the surviving tumor population in partially regressing melanocytic lesions.

434 Diagnostic Value of WT-1 and CD34 in Cutaneous Spindle Cell Neoplasms

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Background: Dermatofibrosarcoma protuberans (DFSP) is an uncommon cutaneous spindle cell neoplasm. Diagnosing DFSP and differentiating it from other cutaneous spindle cell neoplasms and/or proliferations is important due to its malignant potential. Due to similar histologic features in other cutaneous spindle cell lesions, confirmatory immunohistochemical studies may be needed. Co-expression of WT-1 and CD34 has been reported in spindle cell neoplasms such as gastrointestinal stromal tumors. However, the expression and diagnostic value of WT-1 in cutaneous spindle cell neoplasms and/or proliferations has not been investigated. In this study, we evaluated the diagnostic utility of WT-1 and other immunohistochemical markers in cutaneous spindle cell lesions.

Design: 51 cutaneous spindle cell neoplasms and/or proliferations including 16 DFSP, 11 dermatofibroma (DF), 12 neurofibroma (NF), and 12 fibromatosis were retrieved from the hospital computer system. Immunostaining for WT-1, CD117 (c-kit), CD34, and S-100 were performed on an automated immunostainer with appropriate positive and negative controls. Statistical analysis was performed with the Chi-Square method.

Results: Positive immunoreactivity for WT-1 was seen in 14/16 cases (88%) of DFSP, but was not seen in any cases of DF, NF, or fibromatosis. CD34 positivity was seen in 16/16 cases (100%) of DFSP, and 12/12 cases (100%) of NF. All cases of DF and fibromatosis stained negatively for CD34. S-100 staining was positive in 12/12 cases (100%) of NF, but was negative in all DFSP and DF. All cases were negative for c-kit.

Conclusions: Our results indicate that co-expression of CD34 and WT-1 by DFSP is unique in cutaneous spindle cell neoplasms and/or proliferations. We recommend that WT-1 be included in the immunohistochemical panel in the work up of cutaneous spindle cell lesions when DFSP is in the differential diagnosis.

435 Follicular Cutaneous Squamous Cell Carcinoma: An Under-Recognized Neoplasm Arising from Hair Appendage Structures

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Background: Cutaneous squamous cell carcinoma (SCC) with no demonstrable point of epidermal origin is problematic as it raises consideration to metastatic squamous cell carcinoma histologically. There are rare case reports and series of SCC arising from the wall of hair follicle structures. Such lesions have been termed *follicular SCC*.

Design: We prospectively encountered 29 cases of follicular SCC arising in 28 patients over a 15 month period. These cases were selected from a database of 1332 cutaneous SCC's encountered over the same time period by the same authors. Follicular SCC is defined as a cutaneous SCC deriving from a pre-existing hair follicle structure. Lesions were considered to represent 'hybrid' SCC's if an interfollicular epidermal origin was also demonstrated; SCC's in which greater than 50% of the origin was from interfollicular epidermis were excluded.

Results: There were 22 pure follicular SCC's verses 7 hybrid lesions. The male to female ratio was 22:6; the mean age was 75 years (range 53-93 yrs). In the same time period, the authors encountered 1303 primary cutaneous SCC's that derived dominantly from the interfollicular epidermis.

Conclusions: Follicular SCC represents 2.2% of all primary SCC's. Biopsies of such lesions, if the appendage structure of origin is not represented, are histologically indistinguishable from metastatic SCC. Recognition of this under-reported form of SCC is thus essential if an inappropriate diagnosis of metastatic SCC, with potentially harmful and inappropriate therapy and investigation, is to be avoided.

436 TdT Expression in Merkel Cell Carcinoma: Potential Diagnostic Pitfall with Blastic Hematological Malignancies and Expanded Immunohistochemical Analysis

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Background: Merkel Cell Carcinoma (MCC) is an uncommon, aggressive primary cutaneous neuroendocrine carcinoma. Histologically, the differential diagnosis includes the "small round cell" tumor group, particularly metastatic small cell carcinoma and blastic hematological malignancies involving skin/soft tissues; Acute Lymphoblastic Lymphoma (ALL) and Acute Myeloid Leukemia (ALL). Terminal Deoxynucleotidyl Transferase (TdT) is a DNA polymerase which is a sensitive and specific antibody for ALL with a small proportion of AML showing positivity. This study investigates the expression of TdT in MCC by immunohistochemistry along with an expanded immunohistochemical profile which aids in further diagnosis of MCC avoiding potential diagnostic pitfall.

Design: 20 cases with initial diagnosis of MCC were retrieved from the institute database and archival blocks and slides were retrieved spanning 1990-2006. The pathology material was reviewed by a hematopathologist and dermatopathlogist. Immunohistochemistry was performed and graded: 0-no staining, 1+ - <50% staining, 2+ - >50% staining, and clinical information was obtained from patient charts.

Results: After review only 15 cases were confirmed as MCC. (5 males,10 females; age range 54-86 years; extremities (56%), head and neck (19%), trunk (6.7%), Liver and groin metastasis 2 cases). Immunohistochemical positivity was as follows: AE1AE3/CAM5.2 (both membranous and paranuclear dot positivity), NSE, CD56 and Bcl-2 (15/15), Synaptophysin (13/15), Chromogranin A (11/15), CK20 (13/15), CK7 (3/15), CK7and CK20 (3/15), CD99 (2/15), CD117 (8/15). 8/15 cases were positive for TdT with strong nuclear positivity, morphologically resembling blasts (intermediate to large cells with vesicular nuclei and 1-3 nucleoli). All cases were negative for CD45, CD34, CD10, CD20, CD3, Myeloperoxidase and TTF-1 and 1 case was negative for CK7 and CK20.

Conclusions: TdT was expressed in 53% of cases of MCC (not previously reported). MCC expressing TdT show similar morphologic features as blastic hematological malignancies. This can be a potential diagnostic pitfall. In our series, MCC were also positive for CK7 (20%), CD56 and BCL2 (100%), CD99 (13%) and CD117 (53%). 5 cases expressed both TdT and CD117. TTF-1 was negative in MCC. Expanded immunohistochemical panel with positive staining for epithelial/ neuroendocrine markers, CK20 and negative staining for other lymphoid/ myeloid markers with awareness of TdT expression avoids misinterpretation in the diagnosis of MCC.

437 Cutaneous Marginal Zone Lymphoma with Marked Plasmacytic Differentiation and Rare B Cells

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Background: The diagnosis of B-cell lymphocytic infiltrates in the skin can present considerable diagnostic difficulty for the practicing dermatopathologist. Primary cutaneous marginal zone lymphoma (MZL) is classically composed of reactive lymphoid follicles surrounded by an infiltrate of marginal zone B cells. In addition lymphoplasmacytoid cells and mature plasma cells are typically present along with a variable number of centroblasts, immunoblasts, histiocytes and eosinophils. We report four cases with unusual morphology, showing marked plasmacytic differentiation, numerous T cells and few B cells.

Design: Four cases of primary MZL were retrieved from the department of pathology archive and hematopathology consultation files. Immunohistochemistry and in situ hybridization were performed on paraffin-embedded tissue as part of diagnostic work-up.

Results: All patients were male with a mean age of 47 years (range, 20 to 72). Three patients presented with a skin lesion of lower extremity and one had a shoulder nodule. Biopsies showed a dense lymphoid infiltrate, extending into the subcutaneous fat. Numerous plasma cells were seen, including one case with prominent Russell bodies. On review of the immunostains, CD20+ B cells comprised a minor population and were predominantly confined to the follicles, while the vast majority of the cells, some with morphological appearance of marginal zone cells, were CD3+ T cells. Monotypic kappa staining of plasma cells was seen in 4/4 cases. Heavy chain staining was performed on 3 cases and showed that the plasma cells were positive for gamma heavy chain in 2 cases and positive for alpha heavy chain in one case. The small B cells in the follicles stained for IgM and IgD, but not IgG or IgA, supporting their non neoplastic nature.

Conclusions: We present four unusual cases of MZL with marked plasmacytic differentiation and a relative predominance of T cells over B cells. Neoplastic marginal zone B cells were not identified. A prominent neoplastic plasma cell population raises the possibility of a plasmacytoma, however none of the patients had a history of plasma cell neoplasm. Primary cutaneous plasmacytomas are very rare and it is largely believed that cases diagnosed in the past as cutaneous plasmacytoma are in fact MZLs with plasmacytic differentiation. In addition MZL with numerous morphologically atypical T cells should be differentiated from a T cell lymphoma, which is much more prevalent in the skin and has a distinctly worse prognosis.

438 Tetraspanins in Malignant Melanoma

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Background: Tetraspanins or transmembrane 4 superfamily (TM4SF) molecules are superficial transmembrane proteins characterized by four conserved transmembrane domains. Several tetraspanin proteins have been associated with tumor invasion and metastasis in multiple tumor types, including breast and colon cancer. In studies of metastatic melanoma cell lines, expression of CD9 has been shown by microarray to be decreased. In contrast, an increase in expression of CD63 has been related to invasive melanoma phenotypes in vitro. To our knowledge, no study has examined tetraspanin-associated proteins in tissue sections of malignant melanoma.

Design: We studied 40 cases of malignant melanoma: 19 cases without documented metastasis, and 21 with documented metastasis. We stained each case for CD9, CD63, and CD82, using the avidin-biotin complex method, and recorded the percentage of staining. Statistical analysis primarily comprised logistic regression analysis, to test the association between metastasizing melanomas and staining for tetraspanins.

Results: CD9 staining was demonstrated in 74% (25 cases) of primary malignant melanoma. The group mean for CD9 reactivity for the metastatic group was lower (10.3 %) than the non-metastatic group (33.3%). Staining for CD9 was significantly associated with a lower frequency of metastasis (p=0.045). Of the primary cases negative for CD9, 78% were later shown to be metastatic. All of the metastases were negative for CD9. Five of the 6 patients who died of disease showed no staining by CD9. CD63 showed strong staining of all of the melanomas, including multiple histologic types, with no loss in either metastatic or non-metastatic cases. CD82 did not stain any of the malignant melanoma cases.

Conclusions: Absence of tetraspanin CD9 expression is associated with increased metastatic likelihood in malignant melanoma. We found no statistical relationship of CD63 or CD82 expression with metastasis. The tetraspanin CD9 deserves further study to elucidate its potential as a prognostic tool in defining the behavior of malignant melanoma.

439 CD-10 Immunostaining Differentiates Superficial Basal Cell Carcinoma from Cutaneous Squamous Cell Carcinoma

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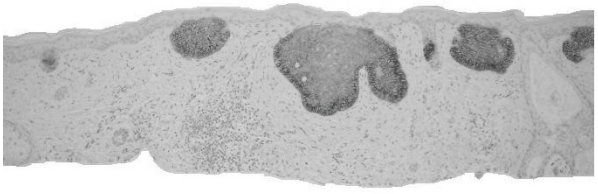
Background: Basal cell carcinoma and squamous cell carcinoma are common entities in clinical practice. Their distinction can be difficult clinically as well as histologically. CD10 or CALLA (common acute lymphoblastic leukemia antigen) is a metallomembrane endopeptidase expressed on a variety of normal and neoplastic cells.

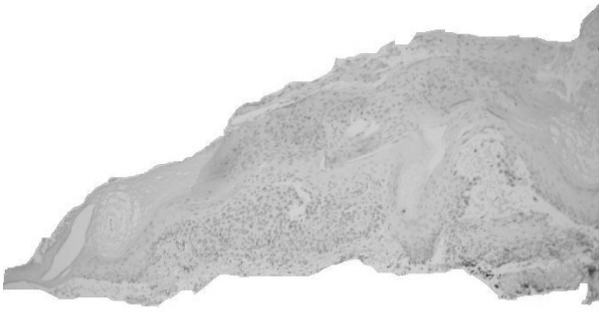
Design: We sought to determine if the CD10 immunostain could have diagnostic utility in distinguishing between early superficial basal cell carcinoma (BCC) and squamous cell carcinoma (SCC).

Results: CD10 was strongly expressed in 14 out of 14 superficial BCCs and failed to express in 2 out of 2 deeply infiltrative BCCs. CD10 was negative in the tumor cells in 13 out of 13 superficially invasive SCCs and SCC in situ. CD10 expressed weakly in the surrounding stromal cells of 2 out of 13 SCCs.

Conclusions: These findings support the utility of CD10 as a marker for early BCC especially when SCC cannot be excluded clinically or by conventional stains. Furthermore, these results implicate CD10 in the pathogenesis of BCC.



Low power photomicrograph depicting intense IHC score (3) staining with CD-10 in a superficial BCC.



Low power photomicrograph depicting a superficial SCC with negative CD-10 expression.

440 Lipodermatosclerosis: A Histological Study of 25 Cases

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Background: Lipodermatosclerosis, also known as sclerosing panniculitis, is a degenerative disease that affects both lower legs. This chronic condition classically affects white females in their sixties.

Design: We collected 25 cases prospectively from our daily practice between September 1998 and December 2005. Patient demographics, lesional characteristics, and clinical information were gathered from submitted specimens and treating dermatologists. All biopsies were stained with H&E, von Kossa and Verhoeff-van Gieson (VVG).

Results: Patient age ranged from 33 to 84 years with an mean age of 62.6 years. There was a strong female predominance with a female to male ratio of approximately 12 to 1. All lesions were present on the lower extremities, at varying sites between the knee and ankle. Lesion duration ranged from 2 months to 2 years with a mean of 9.5 months. Clinically, the lesions were described as erythematous, tender, indurated plaques or nodules. The characteristic histological findings were seen almost exclusively in the subcutaneous tissue, involving primarily the lobules but also the septa. Adipose changes included micro and macrocyst formation, necrotic adipocytes, lipomembranous change and lipogranulomas with xanthomatous macrophages. The lesions were largely devoid of inflammatory changes. Medium vessel calcification was seen in 13 cases. A conspicuous change was the presence of accumulation of basophilic elastic fibers located deep in the septa, present in all of the cases. These fibers had a moth-eaten appearance and resembled the elastic fibers of pseudoxanthoma elasticum. These fibers were positive with both the von Kossa stain and VVG in 21 of the 25 cases.

Conclusions: The constellation of these histological changes is diagnostic of lipodermatosclerosis. While the pseudoxanthoma elasticum –like changes are very characteristic of this condition, they can be rarely seen in other disorders including thalassemia and calciphylaxis.

441 Lymphatic Invasion Revealed by Multispectral Imaging Is Common in Primary Melanomas and Associates with Prognosis

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Background: Lymphatic invasion by tumor cells has been noted infrequently in primary melanomas. Metastasis to regional lymph nodes is more frequent and is associated with poor patient outcomes. We hypothesized that use of a specific immunohistochemical (IHC) marker of lymphatic vessels in primary lesions would increase the frequency of detection of lymphatic invasion and that lymphatic invasion would correlate with regional nodal metastasis.

Design: We studied the primary lesions of a sample of 106 patients from a retrospective cohort of 489 patients with melanomas diagnosed between 1972 and 1991 who had at least 10 years of follow up. We performed IHC stains for podoplanin (a marker for lymphatic vessels) and S-100 (a marker for melanoma cells). Tumoral lymphatic invasion was identified and confirmed by multispectral imaging (MSI) analysis; and tumoral lymphatic density was counted. We computed the rates of detection of lymphatic invasion and presence of intratumoral lymph vessels and used the log-rank test to evaluate differences between the Kaplan-Meier survival curves for time to regional nodal failure.

Results: Using IHC we found that intratumoral lymph vessels were present in 90 (85%) of the 106 primary melanomas. Intratumoral lymphatic invasion was detected by routine microscopy in 5 (4.7%) of the 106 cases and by IHC staining augmented by MSI in 37 cases (35%) ($p<0.001$). Tumoral lymphatic invasion was significantly associated with time to regional nodal metastasis, first metastasis and melanoma-specific death.

Conclusions: Tumoral lymphatic invasion is an under-observed phenomenon in primary melanomas that can be better detected by IHC staining. The presence of intratumoral lymphatic invasion, a step in tumor progression beyond lymphangiogenesis, may be a clinically useful predictor of regionally metastatic disease.

442 Pax-2 Expression in Cutaneous Adnexal Neoplasms

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Background: Pax-2 is a transcription factor that functions in the regulation of epithelial-mesenchymal interactions in developing tissues and organs including skeleton, sense organs, limb muscle, endocrine pancreas, kidney, and brain. In these systems, its expression is down-regulated in terminally differentiated cells. The potential role of Pax-2 in epithelial-mesenchymal signaling in cutaneous adnexal neoplasms has not been previously explored. The regulatory role of Pax-2 in epithelial-mesenchymal interactions during development of other organs suggests a similar role in skin. Based on previous work showing mesenchymal expression in cutaneous adnexal development, it follows that adnexal neoplasms will show Pax-2 expression in a pattern analgous to that seen in renal neoplasms.

Design: Nuclear expression of Pax-2 was evaluated in 31 paraffin-embedded, formalin-fixed adnexal neoplasms: tricholemmoma (6), benign adnexal neoplasm (8), clear cell acanthoma (4), microcystic adnexal carcinoma (4), malignant adnexal neoplasm (2), sebaceous carcinoma (3), proliferating tricholemmal cyst (2), tricholemmal carcinoma (1) and syringoma (1). Metastatic renal cell carcinoma, with known pax-2 expression was used as a positive control.

Results: Scattered nuclei in 9 out of 31 lesions, both benign and malignant, showed strong to weak nuclear Pax-2 expression. This expression was seen in benign adnexal neoplasms (3), sebaceous carcinoma (2), tricholemmoma (1), proliferating tricholemmal cyst (1), clear cell acanthoma (1) and microcystic adnexal neoplasm (1).

Conclusions: Despite mesenchymal induction of overlying epithelium during embryologic development of cutaneous adnaxae and Pax-2 expression in the adnexal mesenchyme in fetal skin, analogous to that seen in renal tubular development, Pax-2 expression does not appear to be upregulated in cutaneous adnexal neoplasms as is seen in renal cell carcinoma. Although parallels in developmental processes and Pax-2 expression exist between renal tubular and cutaneous adnexal formation, this parallel does not carry through to upregulation of Pax-2 in the development cutaneous adnexal neoplasms.

Endocrine

443 Histologic Variants of Papillary and Follicular Carcinomas Associated with Anaplastic Spindle and Giant Cell Carcinomas of the Thyroid: An Analysis of Rhabdoid and Thyroglobulin Inclusions

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Background: Anaplastic spindle and giant cell carcinomas (SGCC) of the thyroid arise from papillary and follicular carcinomas. However, the incidence of the histologic variants of papillary and follicular carcinomas associated with SGCC is unknown. Rhabdoid inclusions have been reported in SGCC and in poorly differentiated follicular carcinoma (PDC) and thyroglobulin inclusions in follicular neoplasms, but the incidences of these inclusions in these tumors are unknown.

Design: A total of 292 thyroid neoplasms were used for this study. One hundred nine were SGCC, 120 papillary carcinomas (PC) (all variants included), 23 differentiated follicular carcinomas (DFC) (6 with insular pattern), 6 PDFC and 34 follicular adenomas (FA). H&E stained sections were available for review in all cases. A specific search for rhabdoid and thyroglobulin inclusions was made in every case. Additional sections were obtained from thyroid neoplasms with cytoplasmic inclusions for immunohistochemical studies using the following antibodies: vimentin, desmin, pancytokeratin, thyroglobulin and calcitonin.

Results: The following differentiated thyroid carcinomas coexisted with SGCC: 51 (46.8%) PC (34 conventional type, 14 tall cell variant and 3 follicular variant), 6 (5.5%) DFC, 1 with insular pattern (0.9%) and 3 oncocytic carcinomas (2.8%). Eleven SGCC (10%) and 2 (33%) PDFC showed rhabdoid features but lacked thyroglobulin inclusions. Thyroglobulin inclusions were found in 10 FA(29%), 8 (17%) follicular variant of PC and 7 (30.4%) DFC. There were no rhabdoid inclusions in any of these differentiated thyroid tumors.

Conclusions: SGCC results from anaplastic transformation of papillary or follicular carcinoma although the mechanisms that underlie this transformation remain unknown. The finding that only 1 SGCC was associated with follicular carcinoma with insular pattern contradicts the opinion that this tumor occupies an intermediate position between differentiated and SGCC. Rhabdoid features are markers of PDFC and SGCC, which are associated with aggressive behavior. Thyroglobulin inclusions reflect functional differentiation, and are markers of FA and DFC with follicular phenotype.

444 Papillary Thyroid Carcinoma, Columnar Cell Variant: A Clinicopathologic and Molecular Study

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Background: Papillary thyroid carcinoma is usually an indolent neoplasm with good biological behavior. However, the columnar cell variant of papillary thyroid carcinoma has a variable clinical course. Encapsulated or well-circumscribed tumors are associated with a favorable prognosis, while others are locally aggressive with early dissemination. The purpose of this study is to correlate the clinicopathologic features of the columnar cell variant with BRAF gene mutations and prognostic markers.

Design: Thyroid specimens included 8 surgical and 1autopsy specimens. The clinical findings and pathology material were reviewed. All specimens met the current WHO classification criteria for this entity. Routine and immunohistochemical stains for cyclin D1, bcl-2, and Ki-67 (MIB-1) were performed. PCR and gene sequencing of BRAF were performed on paraffin embedded tissue.

Results: 9 patients ranged in age from 32 to 90 years and included 5 males (median 60, mean 65) and 4 females (median 38, mean 47). Clinical follow-up was available for 6 of 8 surgical patients. The cases were classified as clinically indolent or aggressive. Indolent tumors included 4 patients (1 male, 3 female; median age 38 years) with asymptomatic or painless masses which were encapsulated or well-circumscribed (1.3 to 4 cm). 2 patients with clinical follow-up had no evidence of residual disease, and 1 patient had an incidental tumor discovered at post-mortem. The remaining 5 patients (4 male, 1 female; median age 60 years) had diffusely infiltrative tumors (4 to 11.5 cm) with extrathyroidal extension, tracheal invasion, and/or metastases. Of this group, 1 patient is alive with disease (25 months follow-up) and 3 died of disease 17 to 45 months after the diagnosis. A $BRAF^{V600E}$ (single letter amino acid) missense mutation was present in 3 of 9 cases, including 2 aggressive neoplasms and the incidental tumor. Cyclin D1 expression was up-regulated in all cases. In both indolent and aggressive tumors, bcl-2 expression was variably decreased and the MIB-1 proliferative index ranged from <5% to 30%.

Conclusions: The $BRAF^{V600E}$ missense mutation was present in 3 of 9 cases, all of which were from older male patients. Interestingly, no detectable BRAF mutation was seen in younger female patients with indolent tumors. There was no significant correlation between indolent and aggressive tumors and expression of cyclin D1, bcl-2, and the MIB-1 proliferative index.