366 HPV Genotypes in 278 Cervical Samples: Evidence for Three Categories of Squamous Intraepithelial Lesions

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Background: LSIL is thought to represent a reversible HPV–associated lesion distinct from HSIL and invasive carcinoma. We have reported that HSIL–moderate (HM) has a different HPV profile from HSIL–severe (HS) and invasive carcinoma (IC). This study adds LSIL cases to an expanded case list.

Design: Liquid–based cytologic specimens from consensus SIL and verified IC cases were blindly analyzed for HPV type using a PCR–based reverse line blot genotyping method (Roche Molecular Systems, Alameda, CA) that identifies 27 HPV types: high risk (HR) types (16,18,31,45); intermediate risk (IR) types (33,35,39,51,52,56,58,59,68,82,83,73); and low risk (LR) types (6,11,26,40,42,43,44,53,54,55,66,84) in a single test. Cases were grouped according to the highest risk HPV type identified in each case.

Results: The previously reported patterns of HPV in HSIL and IC persisted.

Diagnosis (n)	HPV HR (%)	HPV 16 (%)	IR (%)	LR (%)	Multiple HPV (%)
LSIL (63)	18 (27)	10 (16)	30 (45)	15 (22)	38 (60)
HSIL–M (58)	25 (43)	11 (19)	31 (54)	1 (2)	31 (53)
HSIL–S (64)	50 (78)	41 (64)	13 (20)	0 (0)	30 (47)
INV CA (93)	77 (83)	51 (55)	10 (11)	0 (0)	11 (12)

LSIL had two patterns: 1) IR HPV that overlapped with HM; and 2) LR HPV only. χ^2 showed differences of HPV in LSIL vs. HM (p=.002), HM vs. HS (p<.0001) but not HS vs. IC (p=.105)

Conclusions: These data suggest that HS, HM and LSIL have different patterns of HPV types that may reflect differences in biological potential. Only HS had an HPV profile that was indistinguishable from that of IC.

Dermatopathology

367 Loss of S100 Immunoreactivity in Metastatic Melanoma

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Background: In the evaluation of poorly differentiated malignant tumors, metastatic melanoma is chief among the differential diagnosis. The melanocytic marker S100 (reported false-negative rate of less than 4%), is frequently used in such cases to rule-out melanoma. However, difficulty arises in excluding melanoma when S100 is negative.

Design: In an ongoing NCI clinical trial for metastatic melanoma, and over a 5 year period, we identified 17 cases in 1553 patients (1.1%) that were negative for S100 immunostain. Where possible, these cases were also evaluated for other melanoma markers, including HMB45, MART-1, KBA62, tyrosinase and NSE. Additional factors evaluated include the site of the primary lesion, histologic appearance and prior immunostaining profiles for other lesions from the same patient.

Results: All 17 cases of S100-negative melanoma were metastatic lesions. Of these 17 cases, 11 had prior pathologic specimens evaluated for S100 staining, and in 9 of these 11 (82%) cases, there was a prior documented S100 positive metastasis. The time interval for loss of S100 immunoreactivity ranged from 3 weeks to 3 years (average 13.5 months). Ten of the 17 S100-negative lesions (59%) lesions were immunoreactive for both HMB45 and MART-1. Of the remaining 7 cases, four were immunoreactive for NSE (4/7), two were not tested and one was negative for all markers. Fifteen of the 17 S100-negative cases had a documented primary melanoma. Of the 2 cases with no documented primary lesion, both were immunoreactive for HMB45/MART1. Interestingly, there was a disproportionate representation of ocular melanoma (4 of 17, 23.5%) while primary ocular melanoma represents approximately 2.0 % of all melanomas. There was no association between S100-negative status and histologic appearance or site of metastasis.

Conclusions: Approximately 99% of our documented metastatic melanomas are immunoreactive for S100. In our series, 65% of S100-negative melanoma lesions had a prior specimen that was immunoreactive for S100, suggesting either de-differentiation or clonal selection. Furthermore, immunoreactivity for HMB45/MART1 or a history of prior melanoma plus NSE immunoreactivity identified all S100-negative metastatic melanomas. Because 82% of S100-negative melanomas were previously S100-positive, extensive sampling (or multiple biopsies over time) of undifferentiated tumors may increase the possibility of diagnosing metastatic melanoma.

368 Superficial Malignant Peripheral Nerve Sheath Tumors: A Rare and Challenging Diagnosis

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Background: Malignant peripheral nerve sheath tumors (MPNSTs) are generally regarded as sarcomas that occur in the deep soft tissues. Rarely, primary MPNSTs with a cutaneous or subcutaneous (superficial) origin have been reported. We present the clinicopathologic features of 5 cases of MPNST presenting in superficial locations.

Design: Five cases of primary MPNST with a superficial origin were identified and their clinicopathologic features were reviewed. Immunohistochemical studies (IHC) included S-100 protein, neurofilament, CD34, pan-cytokeratin, EMA, SMA, desmin, HMB45, MelanA, tyrosinase, GFAP and microphthalmia transcription factor (MITF).

Results: Four females and 1 male, 18-74 years of age (median = 27) were included in the study. One patient carried a diagnosis of type I neurofibromatosis. The lesions arose in the hip/thigh region (2 cases), distal thigh/knee, wrist and neck. The tumor sizes ranged from 2.6 to 16 cm (median = 3 cm). Four cases appeared to arise from cutaneous neurofibromas and one case was closely associated with a superficial peripheral nerve. Four cases had classic spindled morphology and one was epithelioid. Two cases had areas of heterologous differentiation; one with rhabdomyosarcoma and angiosarcoma and one with osteosarcoma and chondrosarcoma. Mitotic figures ranged from 2 to 50 per 10 hpfs (median = 10/10 hpfs). The four cases with spindle cell morphology were at least focally positive for S-100 protein, while the associated benign neural elements had more extensive S-100 protein immunoreactivity. The epithelioid tumor was diffusely and strongly positive for S-100 protein. Melanoma markers, EMA, GFAP, neurofilament, pan-cytokeratin, CD34, SMA and desmin were negative in all cases with the exception of the areas of heterologous differentiation, which stained with antibodies appropriate to their histologic type. All were treated with wide local excision, one received chemotherapy, and one radiation. There were no local recurrences but three patients died of metastatic disease within 2 to 30 months (median = 12 months).

Conclusions: Superficial MPNSTs are rare. Immunohistochemical markers are helpful in excluding other lesions in the differential diagnosis including malignant melanoma, dermatofibrosarcoma protuberans, leiomyosarcoma and metaplastic carcinoma. However, identification of a benign precursor or origin from a nerve may be the most definitive way to properly classify these rare and challenging lesions.

369 Three-Dimensional Modeling of Pigmented Lesions Can Distinguish between Benign and Malignant Melanocytes

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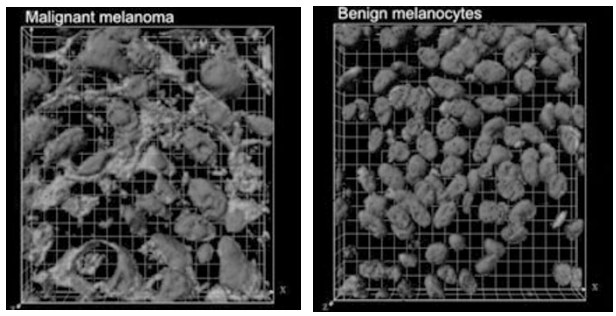
Background: Using the gold-standard of light microscopy, determining the biologic potential of melanocytic lesions may occasionally be difficult and subjective. Despite recent advances, adjunct molecular tests are not yet available to conclusively resolve difficult lesions. We believe that valuable information remains to be discovered using confocal laser scanning microscopy (CLSM) whereby 3-dimensional (3-D) surface renderings of benign and malignant melanocytes can be compared.

Design: Five-micron sections of formalin-fixed, paraffin-embedded benign and malignant melanocytic lesions were deparaffinized, rehydrated and stained with acridine orange (RNA and DNA stain) and DAPI (DNA stain). These lesions were then visualized using a Zeiss LSN 510 confocal laser scanning microscope (0.2-micron intervals). The scanned images were then reconstructed into a 3-dimensional surface rendering using Imaris v4.03 software. Because the intensity of each lesion is normalized to an internal control (nuclear nucleic acid), only the nuclear (DNA + RNA) and cytoplasmic (RNA) surface outlines are used in the analysis.

Results: 3-D surface projections of nuclei and cytoplasm were compared among 8 malignant and 8 benign melanocytic lesions. As expected, malignant nuclei were larger and more irregular than benign nuclei. Notably, in contrast to the more uniform cytoplasmic distribution of RNA in benign cells, the malignant cytoplasmic phenotype is characterized by hierarchical and circumferential branching.

Conclusions: We believe that valuable information on the biologic potential of difficult melanocytic lesions can still be extracted from the standard 5-micron tissue section. While nuclear atypia of melanoma has previously been recognized, we report that melanoma appears to have a more prominent and characteristic cytoplasmic distribution of RNA than do benign melanocytes, perhaps reflecting a higher metabolic rate. We are currently studying whether 3-D surface renderings of atypical (dysplastic) nevi provide diagnostically relevant information.



370 Study of Translocation t(11;18)(q21q21) (API2/MALT1) and Expression of Bcl-10 in Primary Cutaneous Marginal Zone B-Cell Lymphomas

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Background: Primary cutaneous marginal zone B-cell lymphoma (C-MZL), an usually benign low-growth fraction neoplasm, probably is the most frequent form of primary B-cell lymphoma of the skin. API2-MALT1 fusion transcripts due to t(11;18)(q21q21) and aberrant bcl-10 nuclear expression have been described in some noncutaneous marginal zone lymphoma subtypes, but little is known about cutaneous-MZL genetic alterations. Our aim was to evaluate a possible implication of API2-MALT1 fusion transcripts and bcl-10 expression in primary cutaneous-MZL.

Design: A total of 42 patients diagnosed with cutaneous-MZL on the basis of WHO/EORTC criteria were studied. Skin biopsies from every patient were routinely tested with a wide panel of monoclonal antibodies. Additionally, aberrant nuclear bcl-10 expression was evaluated in all cases and RT-PCR amplification with specific API2-MALT1 fusion transcript primers was performed in 21 instances.

Results: Aberrant nuclear bcl-10 expression, demonstrated in 15 cases, was related to a more aggressive clinical behavior. Translocation t(11;18)(q21;q21) was absent in all evaluated samples.

Conclusions: The API2-MALT1 fusion, strongly associated with extracutaneous MALT lymphomas, does not seem to play a role in cutaneous marginal zone lymphoma pathogenesis. However, bcl-10 aberrant nuclear expression could be a significant prognostic factor in cutaneous marginal zone lymphoma, signaling a more aggressive clinical course and a higher risk of extracutaneous involvement.

371 E-Cadherin Expression in Primary Malignant Melanoma with and without Sentinel Lymph Node Metastasis

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Background: E-cadherin is a calcium-dependent transmembrane glycoprotein, primarily serving as an adhesion molecule. Disruption of E-cadherin expression has been implicated in tumor progression and metastasis. Reduced E-cadherin expression has been demonstrated in human malignant melanoma (MM) cell lines. However, *in vivo* studies of E-cadherin expression in MM yield conflicting results. The aim of our study was to analyze the expression pattern of E-cadherin in primary melanomas (PMs) and corresponding metastatic sentinal lymph nodes (SLN).

Design: We analized 32 PMs, all of them with SLN biopsy. The PM types were: 15 superficial spreading (SS), 12 nodular, 4 acral lentiginous (AL) and 1 lentigo malignant melanoma (LM). 7/32 cases were associated with SLN metastasis. All PMs and 6 SLN were analized by IHC for E-cadherin expression. The membranous expression of E-cadherin was semiquantitatively estimated using three categories: negative, none to less than 5%; focal, 5-50%; and strong diffuse, more than 50% positive cells.

Results: Strong diffuse membranous E-cadherin expression was present in 16/32 cases, 11 of which were SS (69%), 4 nodular (25%) and 1 AL type (6%). Focal expression was seen in 14/32 cases, 6 nodular, 4 SS, 3 AL and 1 LM type. Two nodular PMs were completely negative for E-cadherin. 14/25 PMs without SLN metastasis showed strong E-cadherin expression (56%), 9 showed focal expression (36%) and 2 cases were negative. 2/7 PMs with SLN metastasis showed strong immunopositivity (29%) and focal expression of E-cadherin was seen in the remaining 5 cases (71%). The median thickness of PMs was 3.4 mm in cases with strong E-cadherin expression, 4.9 mm in cases with focal and 6.1 mm in negative cases. 1/6 metastatic SLN was strongly positive for E-cadherin, 4 were focally positive and 1 was negative. All metastasis showed decreased E-cadherin expression when compared to their corresponding PMs.

Conclusions: Our results indicate that expression of E-cadherin in PMs correlates with growth pattern with strong expression predominantly seen in the SS type. The expression of E-cadherin seems to decrease with increase in thickness of PMs. In addition PMs with metastatic SLN show decreased expression of E-cadherin when compared to PMs without metastatic SLNs. The further attenuation of expression in the metastasis compared to their corresponding primary lesion suggest that E-cadherin may play a role in the progression of the disease.

372 Differential Mutational Activation of the BRAF Oncogene in Desmoplastic and Non-Desmoplastic Cutaneous Melanomas

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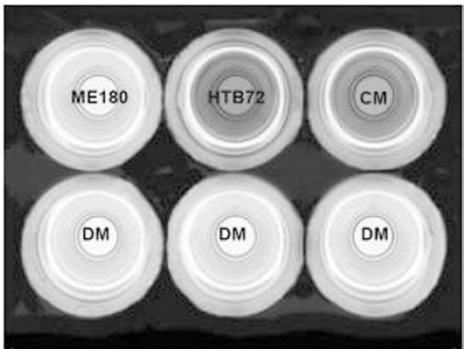
Background: Desmoplastic melanoma is an uncommon variant of cutaneous melanoma that mimics soft tissue sarcoma both clinically and morphologically. An activating thymine → adenine missense mutation at nucleotide 1796 (T1796A) of the BRAF oncogene has been identified in a high proportion of conventional cutaneous melanomas, but its frequency in the desmoplastic subtype is not known.

Design: We tested 57 conventional vertical growth phase melanomas and 12 desmoplastic melanomas for the BRAF T1796A mutation using a newly developed, mutation-specific primer extension technology (Mutector® assay). The ME180 cell line (wild-type BRAF) and the HTB72 melanoma cell line (homozygous for BRAF T1796A) served as controls.

Results: Using the Mutector® assay, the presence of a BRAF mutation is indicated by a measurable colorimetric change from clear to green. In the figure below, a mutation is detected in the HTB72 control and a representative conventional melanoma (CM), but not detected in the ME180 control or three desmoplastic melanomas (DM). BRAF mutations were detected in 23/57 conventional melanomas and 0/12 desmoplastic melanomas (p=0.0006).



Summary of Clinicopathologic Features and BRAF Status for Desmoplastic and Non-desmoplastic Melanomas

Melanoma type	Site	Patients (n)	Mean age (yrs)	Caucasian (%)	Male : Female	Mean depth of invasion (mm)	BRAF mutation n (%)
Desmoplastic	Head and neck	11					0 (0)
	Trunk	1					0 (0)
	Total	12	54	100	6 : 6	14.1	0 (0) *
Non-desmoplastic	Head and neck	20					5 (25)
	Trunk	17					7 (41)
	Extremity	17					11 (65)
	Acral	3					0 (0)
	Total	57	59	91	30 : 27	3.1	23 (40) *

* p = 0.0006, Fisher's exact 2-tailed test

Conclusions: In contrast to conventional cutaneous melanomas, the desmoplastic variant does not frequently harbor an activating mutation of BRAF. Distinct genetic alterations may underlie well recognized clinical and morphologic differences among melanoma subtypes. Accordingly, patients with melanoma should not be regarded as a uniform group as new therapeutic strategies are developed that target specific genetic alterations.

373 p63 Is a Useful Marker for Cutaneous Spindle Squamous Cell Carcinoma

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Background: p63 is a member of the p53 gene family. In the skin, it is expressed in the nuclei of basal and spinous cells of the epidermis, peripheral cells of the eccrine dermal ducts, germinative cells of sebaceous glands, myoepithelial cells of the terminal portion of the eccrine glands and aprocrine glands. In contrast to the tumor suppressive function of the p53, over-expression of p63 in many squamous cell carcinomas suggests that it could act as an oncogene.

Cutaneous spindle squamous cell carcinoma (spindle SCC), also known as metaplastic carcinoma, is a rare variant of SCC. This lesion is sometimes difficult to diagnose based purely on morphologic features. Its differential diagnosis includes other spindle cell malignancies including atypical fibroxanthoma (AFX), spindle cell melanoma, and dermatofibrosarcoma protuberans. The results of p63 staining have not been previously reported in this differential diagnosis.

Design: In a ten-year period (1994-2004) eleven cases of spindle SCC were diagnosed at our institution. Clinical features of each patient with spindle SCC were gathered. All spindle SCC and controls were stained with DAKO^{R} antibodies: p63, CK 903, MNF 116, and vimentin. Control cases used were desmoplastic melanoma (7 cases), atypical fibroxanthoma (5 cases), and dermatofibrosarcoma protuberans (2 cases).

Results: Of spindle SCC patients, the mean age was 75 years (range 42-97 years), and the mean tumor size was 16 mm (range 2-41 mm). All of these carcinomas occurred in sun-exposed areas, the head and neck region being the most common location.

p63 was expressed diffusely in the nuclei in 100% (11/11) of the spindle cell SCCs. All controls were negative for p63. Epidermal basal cells and adnexal structures served as positive internal controls. MNF116, CK903, and vimentin were also positive in the cytoplasm of all spindle cell squamous cell carcinomas. All control cases were positive for vimentin and negative for cytokeratins. All desmoplastic melanomas were positive for S100.

Conclusions: In the differential diagnosis of cutaneous spindle cell malignancies, p63 appears specific for spindle cell SCC and adds a useful nuclear marker to the repertoire used in this differential.

374 High Levels of Vascular Endothelial Growth Factor Receptors 2 and 3 (VEGFR2 and VEGFR3) Correlate with High Grade in Cutaneous Angiosarcomas

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Background: Expression of VEGF and its receptors is upregulated in a variety of vascular tumors. Of the several signal transduction pathways activated by VEGF binding, p38 mitogen-activated protein kinase (MAPK) is critically involved in cell cycle control and endothelial cell migration. Specifically, MAPK causes prostaglandin synthase upregulation leading to PGE2 production and stimulation of tumorigenesis and angiogenesis.

Design: We have immunohistochemically examined the expression of p38, VEGFR2, VEGFR3, mPGE synthetase 1, mPGE synthetase 2, cyclooxygenase 2, p53, and Ki67 in 12 benign vascular tumors and 9 angiosarcomas (5 high-grade, 4 low-grade) of the skin.

Results: Expression of Ki67 was significantly higher (p=0.008) in the angiosarcoma group than in benign vascular lesions. When compared with their low-grade counterparts, high-grade angiosarcomas showed significantly higher VEGFR2 (p=0.016) and VEGFR3 (p=0.016) expression levels and displayed a trend to higher Ki67 and p53 levels. VEGFR2 and VEGFR3 overexpression (>50% of positive tumor cells) was not observed in any case of low-grade angiosarcoma, but was present in 80% and 100% of high-grade cases, respectively. All instances of low-grade angiosarcoma were devoid of p53 immunoreactivity.

Conclusions: In angiosarcomas, high grade is associated with elevated VEGFR2 and VEGFR3 overexpression, which in turn tends to correlate with high Ki67 expression levels, even in cases showing no evidence of p53 overexpression. These data suggest that VEGFR2 and VEGFR3 overexpression may be involved in malignant progression in angiosarcoma.

375 Endocrine-Mucin Producing Sweat Gland Carcinoma of the Eyelid: Analysis of 12 Cases Suggests That It Is a Precursor of Mucinous Carcinoma

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Background: Endocrine mucin-producing sweat gland carcinoma (EMPSGC) is an under-recognized low grade eyelid carcinoma with only three cases described in the literature. This tumor shares some morphological features with papillary solid carcinoma of the breast, which is frequently associated with mucinous carcinoma. Here, we describe 12 new cases of EMPSGC which suggest that EMPSGC is a precursor of some mucinous carcinomas of the eyelid.

Design: Histological analysis of hematoxylin & eosin stained sections and immunohistochemical studies with chomrogranin,synaptophysin, neuron specific enolase (NSE), estrogen receptor (ER), progesteron receptor (PR), EMA, CEA, cytokeratin 7 (CK7), cytokeratin 20 (20), and Cam5.2 antibodies of 15 biopsies from 12 patients was performed. Retrospective analysis of clinical data was also done.

Results: EMPSGC were more frequent in females than males (5:1) with an average age of 69 years (33-84). All tumors occured as slowly growing nodules on the eyelids. Clinical follow-up showed no recurrences. EMPSGC showed features as previously described (Flieder et al. Am. J. Surg. Pathol., 1997, 21, 1501-1506). The tumors were well circumscribed, solid or partially cystic nodules of small to medium sized oval to polygonal epithelial cells with abundant cytoplasm, and bland oval nuclei with inconspicuous nucleoli, which formed solid sheets and compressed paillary structures. Intra and extracellular mucin was usually present. Mitotic activity was rare. EMPSGC showed a consistent immunophenotype with the expression of neuroendocrine markers (synaptophysin, chromogranin or NSE), ER, PR, CK7, EMA, CEA, but not CK20. EMPSGC was associated with tumor cells involving benign eccrine ducts in multiple cases. The histological spectrum of intraductal tumor ranged from a flat, single cell layer proliferation undermining benign eccrine epithelium to clinging and micropapillary carcinoma in situ, which also showed neuroendocrine differentiation. In 9 cases, areas of classic invasive mucinous carcinoma were present.

Conclusions: The series provides histologic evidence for a multistage progression of eccrine neuroendocine carcinoma in situ to EMPSGC and then to mucinous carcinoma of the eyelid. Although the data from this series supports the notion that the prognosis of EMPSGC is good, longer follow-up is needed for better understanding of their pathogenesis and clinical behavior.

376 Type VII Collagen in Alport Syndrome

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Background: Absence or segmental distribution of α_5(IV)chain along the dermo-epidermal junction (DEJ) is currently considered to be diagnostic of X-linked Alport syndrome (X-AS), although typical morphologic alterations of the basement membrane (BM) are lacking. However, several additional collagenous components are present in the DEJ and their assembly is likely to be disturbed by the absence of α5(IV). Collagen VII is present in large amounts in the DEJ, where it is instrumental in maintaining BM integrity. The purpose of this study was to investigate the distribution of Collagen VII in the DEJ of patients with X-AS.

Design: Skin biopsies were obtained from 5 cases of X-AS with absence of α(IV), 5 cases with segmental α_5(IV) and 5 controls. 5μ thick cryostat sections of frozen tissues were incubated with antibodies against Laminin-1 and Collagen VII, followed by FITC-conjugated secondary antibodies. Sections were examined with a confocal microscope: a single horizontal scan was performed at the plane of highest intensity of the signal and area occupied by the fluorochrome was calculated by pixel count and expressed in μ^2. All scans were performed using the same objective (60X). Quantitative analysis for Collagen VII was also performed by Real Time-PCR.

Results: Confocal microscopy demonstrated that the area occupied by type VII collagen within the basement membrane zone was constantly higher in the skin samples from X-AS patients than in controls. By RT-PCR, type VII collagen mRNA was detected in significatively higher amounts in all the skin fragments in which α_5(IV) chain was immunohistochemically absent. Segmental distribution of α_5(IV) was associated with mRNA expression that ranged from slight increase to high values, when compared with control samples. The area occupied by laminin was not different among the three groups.

α_5(IV)	Coll. VII (μ^2)	Laminin-1 (μ^2)
Controls	21.44±8.26	7.17±1.27
Absent	41.19±2.77	7.07±1.01
Segmental	35.9±8.52	6.69±0.57

Conclusions: Our results showed for the first time that absence of α_5(IV) is associated with increased amounts of Collagen VII vs. controls ($p<0.001$), indicating that lack of one of the chains of Collagen IV molecule significantly alters the assembly of other extracellular matrix molecules also at the DEJ level. We suggest that the increased synthesis and deposition of Collagen VII is likely to balance the absence of stabilizing activity normally exerted by α_5(IV).

377 The Use of Immunohistochemical Studies in the Analysis of Sentinel Lymph Nodes in Patients with Melanoma—Is it Necessary? An Eight Year Follow-Up Study

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Background: Lymph node metastasis is an important predictor of survival in the staging of cutaneous melanoma. Detection of lymph node metastases also provides valuable information for treatment, thus the identification is integral to the current staging and management of melanoma. In many centers the current standard of care for high-risk clinically node-negative melanoma is sentinel lymph node biopsy (SLN). SLN biopsy allows accurate sampling of selected lymph nodes with minimal morbidity. The ability of the SLN biopsy techinique to detect the smallest amount of tissue with the highest probability of metastatic localization has prompted many to question the approach to pathologic examination of lymph nodes. Multiple lymph node sections coupled with immunohistochemistry (IHC) have been shown to improve detection of metastases. However, studies comparing improved detection with clinical outcome are necessary. We hypothesize that multiple lymph node sections and IHC stains will offer greater detection of micrometastases within SLN.

Design: 80 SLN from 50 cases of melanoma in which SLN metastases were not detected by conventional pathologic examination were evaluated. All of the SLN were re-examined by step-sectioning at five micron (μ) intervals, taking four consecutive sections at 125μ and 475μ. One section from each level was stained with each of the following: H&E, HMB45, S-100 protein, and MART-1. Detection of SLN melanoma metastases was based on IHC staining and morphology.

Results: 1 of 80 SLN samples demonstrated evidence of micrometastasis at 475μ after staining with S-100 and HMB45. Review of an accompanying H&E slide cut at the same level indicated that recognition of the micrometastasis would have been problematic without the IHC staining data because of its subtle morphology.

Conclusions: The data shows that IHC with additional sections only minimally improves detection of SLN micrometastasis. The low prevalence of increased detection raises the issue of practicality of routine IHC screening at multiple levels. Additional studies are in progress on an additional 68 patients to determine the prevalence of micrometastasis identifiable after IHC assay and to determine the outcome in this patient population with a median follow-up of 8 years in relation to micrometastatic tumor burden.

378 Use of Chromogenic In Situ Hybridization (CISH) To Determine Loss of Melastatin Expression in Sentinel Lymph Node Negative Melanoma

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Background: Tumor depth remains the most important indicator of metastatic potential in invasive melanoma, and there is no cure for patients who develop metastases. Adjuvant therapy is reserved for patients with confirmed lymph node metastases. However, some patients with thin tumors and negative sentinel lymph nodes (SLN) develop metastases despite a seemingly good initial prognosis. Melastatin (MLSN) is a gene that is expressed uniformly in benign nevi, and shows more variable expression in melanoma. Loss of MLSN expression has been shown in some studies to correlate with poorer disease-free survival.

Design: We retrospectively examined 30 cases of melanoma with Breslow depths ranging from 0.93 to 17mm. SLN sampling performed in each case showed no metastatic disease with H&E, S100 protein, and HMB45 stains. All patients subsequently developed metastatic disease and were either alive with disease (AWD) or dead of disease (DOD) at the time of the study. We used CISH to evaluate MLSN status in the original biopsies and to determine whether the loss of MLSN correlated with aggressive disease.

Results: All cases showed at least partial loss of melastatin expression. There was wide variation in MLSN expression by tumor stage and by follow-up status (AWD vs. DOD), with no definitive increased loss of MLSN with higher stage. However, early stage I melanomas showed a high loss of MLSN compared to stage II (65% vs 34% loss). Pairwise covariance analysis applied to histologic staging parameters showed that increasing loss of MLSN expression significantly correlated with presence of microsatellites and increasing depth (r values of 0.4125 and 0.3705, respectively).

Conclusions: Most patients diagnosed with melanoma present with stage I or II disease. MLSN expression used in conjunction with AJCC staging may allow identification of a subset of patients with more aggressive disease, thereby permitting the use of adjuvant therapy prior to development of documented metastatic disease, regardless of SLN status.

379 Sentinel Lymph Nodes Are Immunosuppressed Whether or Not They Contain Metastatic Melanoma

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Background: Immune suppressed lymph nodes show reduced high endothelial venule (HEV) frequency and expression of CD 105 ($CD105^+$HEV) by HEV and reduced transmigration of activated $OPD4^+$T cells through these vessels. We investigated whether nodal immunosuppression requires the physical presence of metastatic melanoma in nodes.

Design: Paired sentinel (SN) and non-sentinel nodes (NSN) from 35 melanoma patients (14 with nodal metastases and 21 with no metastases in SN) were evaluated by double staining immunohistochemistry: CD105 for HEV and OPD4 for activated T cells. We divided the nodal section into 4 quarters and captured 5 images from each quarter for analysis using the Simple-PCR image system.

Results: We evaluated overall nodal activation using the density of $OPD4^+$activated T cells. There was no difference in $OPD4^+$T cell density between SN with and without metastases (1295.7±581.28/mm^2 and 984.7±564.98/mm^2, $p=0.111$), but activated T cells were less abundant in all SN than in NSN (1141.3±556.2/mm^2, 1451.1±588.7/mm^2, $P=0.001$). We compared SN with and without nodal metastases by measuring the density and nodal area (%) occupied by $CD105^+$HEV and $CD105^+$HEV with $OPD4^+$T cell transmigration. Significant differences were not found

in these comparisons. In SN and paired NSN, $CD105^+$HEV area (%) relative to measured nodal area was 3.2±3.37% in SN and 6.5±4.44% in NSN, P=0.0005. $CD105^+$HEV density was 15.9±14.15/mm^2 in SN and 27.7±16.21/mm^2 in NSN, P=0.001. The intensity of CD105 expression by endothelial cells was measured using mean greylevel. Lower intensity (higher greylevel number) was found in SN relative to NSN (148.1±11.94 vs. 142.3±13.01, P=0.024). The area (%) occupied by $CD105^+$HEV with $OPD4^+$T cell transmigration was 0.6±0.44% in SN and 1.8±1.76% in NSN, P=0.0002. The density of $CD105^+$HEV with $OPD4^+$T cell transmigration was 2.2±2.80/mm^2 in SN and 4.9±4.37/mm^2 in NSN (P =0.007).

Conclusions: SN in melanoma patients are immune suppressed whether or not they contain metastatic melanoma. Immunosuppression may be modulated by cytokines or other cell products derived from intra-nodal metastatic or extra-nodal (primary) melanoma. Reduced CD 105 expression by high endothelial cells of post capillary venules and reduced trans-venular T cells migration in SN index immune down-regulation, in line with previously described alterations in antigen processing cells. Tumor-induced immune down-regulation of SN may determine their susceptibility to establishment of metastatic melanoma.

380 "Stealth" Melanoma Cells; the Basis of Histology-Negative PCR-Positive Sentinel Nodes?

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Background: Histopathologically negative sentinel nodes (SN) reportedly contain occult melanoma metastases identifiable by sensitive molecular approaches (RT-PCR). There is controversy around the significance of these findings as the cellular source of such augmented signals cannot be visualized microscopically. The reverse transcriptase *in situ* polymerase chain reaction (RT *in situ* PCR) can amplify small amounts of melanoma-specific mRNA and allows identification of cells that contain the amplified mRNA.

Design: Formalin-fixed, paraffin-embedded melanoma-free SN and non-sentinel node (NSN) pairs and tumor-free NSN from patients with a positive SN were evaluated. One step RT-PCR was performed using polymerase r*Tth* to amplify melanoma-restricted MART-1 mRNA *in situ*. Amplified products were detected by a digoxigenin-alkaline phosphatase system with NBT/BCIP.

Results: Cells expressing MART-1 mRNA were detected in 4/15 SN (27%) and 2/13 NSN (15%) that were negative on HE and immunohistology. While some cells that contained MART-1 mRNA diffusely present in the cytoplasm appeared to be melanoma cells others were macrophage-like. These cells expressed augmented product on granules that were mixed with melanin granules. In other cases macrophage-like cells contained nuclei and nucleoli more typical of melanoma cells and may represent the macrophage-melanoma hybrids that have been reported.

Conclusions: Histology and immunohistology-negative lymph nodes contain cells that express mRNA for the melanoma marker MART-1. Some of these cells appear to be "stealth" melanoma cells. Others appear not to be melanoma cells and this should be taken into account when interpreting and applying the results of RT-PCR analysis of nodal and other tissues. CA29605

381 PNL2 - Immunohistochemical Characterization of a Novel Melanocyte Differentiation Marker

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Background: PNL2 is a new monoclonal antibody for the diagnosis of cells and tumors of melanocyte differentiation. However, no comprehensive analysis of its reactivity pattern has been done yet. In the present study, we analyzed the PNL2 immunoreactivity in various panels of normal tissues, melanocytic and related lesions, non-melanocytic tumors, especially histological mimics of melanoma, as well as multiple cell lines.

Design: The IHC analysis of PNL2 (DAKO, Carpentaria, CA) was done on standard archival paraffin tissues using an ABC detection system. Conventional sections from whole tissue blocks as well as tissue micro-arrays were employed.

Results: PNL2 stained normal melanocytes. Staining was also present in neutrophils in various locations, but in no other normal cells. 15 compound, 15 dysplastic, and 2 blue nevi were all PNL2 positive. In compound nevi, staining was most prominent in the dermoepidermal junction. All 13 primary epitheloid malignant melanomas (mM) were positive and 4/5 pleomorphic spindle cell mMs showed heterogeneous staining. Only 1/13 desmoplastic mM was PNL2 positive. In metastatic mM, PNL2 was present in 33/38 (87%) tumors. None of the histological mimics of mM (neurofibroma/5, cellular dermatofibroma/5, AFX/2, cellular neurothekoma/2) were positive except scattered cells in 1/13 MPNSTs. 10/10 angiomyolipomas were also PNL2 positive. All non-melanocytic tumors (30 carcinomas, 3 sarcomas) were PNL2 negative. Furthermore, 5 acute myelogenous leukemia (AML) and 3 chronic myelogenous leukemia (CML) cases were studied because of PNL2 reactivity in neutrophils: all AML cases were negative while 3/3 CML cases were PNL2 positive solely in mature myeloid cells. Of 38 cell lines tested 5/8 mM cell lines were positive, the remaining 30 cell lines were negative.

Conclusions: PNL2 appears to be a valuable new reagent for the diagnosis of cells and tumors of melanocytic lineage and related lesions. Its reactivity pattern is similar to other melanocyte differentiation markers such as A103, HMB45 or T311 showing a high sensitivity in metastatic and primary melanoma though desmoplastic mM remains a problem. PNL2 reactivity in neutrophils needs to be considered but should not cause diagnostic confusion. Since the PNL2 epitope has not been cloned yet, no comparative analysis of its protein and mRNA expression is possible and hence no conclusion regarding potential cross-reactivity or its value as a target for immunotherapy can be drawn yet.

382 Fatty Acid Synthase Expression in Melanocytic Lesions

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Background: Mammalian fatty acid synthase (FASE) is a multifunctional enzyme complex involved in *de novo* synthesis of saturated fatty acids, and inhibitors of FASE are being evaluated as potential therapeutic agents. Increased FASE expression has been demonstrated in subsets of malignancies and melanomas, but has not been investigated in the entire gamut of melanocytic lesions.

Design: FASE expression in 155 melanocytic lesions was scored based on intensity and percentage of positive cells (0-3). Analysis of variance with Bonferroni corrections for multiple comparisons was performed.

Results: Of the primary melanomas (Clark level I:10 cases, II:10, III:9, IV:10, V:9), mean FASE scores were significantly greater for Clark levels III and IV compared to levels I and II ($p < 0.001$). Also, melanomas with Breslow thickness > 1.5 mm showed significantly higher mean FASE scores compared with those < 0.75 mm.

Tumor type	N	Mean+SD	Significant difference between following group pairs ($p < 0.001$)
Conventional nevi (CN)	19	0.21+0.42	CN:CgN, CN:M, CN:MetM
Spitz nevi (SN)	40	0.5+0.72	SN:CgN, SN:M, SN:MetM
Congenital nevi (CgN)	30	2.25+0.57	CgN:CN, CgN:SN, CgN:M
Primary melanoma (M)	48	1.26+0.95	M:CN, M:SN, M:CgN, M:MetM
Metastatic melanoma (MetM)	18	2.56+0.59	MetM:CN, MetM:SN, MetM:M

Conclusions: Our results show that melanomas exhibit stronger FASE expression in comparison to conventional and Spitz nevi. The FASE expression in melanoma increases with greater depth of invasion, and is the highest for metastatic melanoma. Of interest, congenital nevi also show strong FASE expression, similar to that seen in metastatic melanoma. Since normal fetal tissues are known to express high levels of FASE, this may represent a regression to a fetal phenotype.

383 Mucinous Carcinoma of the Skin, Primary and Secondary: A Study of 55 Cases Demonstrating a Morphologic Spectrum of Primary Cutaneous Mucinous Carcinoma Analogous to Mucinous Lesions in the Breast

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Background: Literature suggest microscopic heterogeneity of primary mucinous carcinoma similar to mammary lesions.

Design: We retrospectively studied 55 cases of primary and secondary cutaneous mucinous carcinoma. Clinical workup and presence of in situ component (preserved myoepithelial cell layer) were used to establish origin. Cases were studied microscopically, immunohistochemically and ultrastructurally. When present, in situ component was classified as ductal hyperplasia (DH), atypical ductal hyperplasia (ADH) or ductal carcinoma in situ (DCIS), using criteria of Rosai (2004).

Results: Origins included skin (n=33), breast (n=9), GIT (n=9) and others (n=4). **Skin primary**: there was female predominance (20:13); median age was 65 yrs; locations included scalp (n=13), face (n=8), eyelid (n=7) and others (n=5). Lesions were solitary nodule or cystlike lesions (0.5 to 5 cm). Follow-up: NED (n=14) and recurrence (n=5). Microscopically, 29 cases were pure colloid carcinomas and 4 cases showed invasive ductal component (mixed type). In situ component was seen in 18 cases of pure type and in all 4 cases of mixed type. In pure type, in situ lesions were represented by DCIS, ADH, or DH alone or by spectrum of changes ranging from normal epithelium to DCIS, while in mixed type they were all DCIS. Some lesions had mucocelelike configurations. In cases with in situ lesions there was evidence of cell detachment into lumen from underlying myoepithelial cells or basal membrane; detached cells then became free-floating nests. EM and IHC (antibodies to MUC1, MUC2) studies inferred phenomenon of inverse polarity. **Mammary mucinous carcinoma involving skin**: all patients presented with lesions on chest wall, breast, axilla, and this can serve as clue to breast origin. Microscopically, cutaneous lesions were of both pure and mixed type, and this correlated with primary in breast. **Intestine carcinoma involving skin:** locations included abdomen, leg, and anogenital area. Microscopically, so-called dirty necrosis was seen in 8 cases and thus can serve as clue to gut origin.

Conclusions: Primary cutaneous mucinous carcinomas span morphologic spectrum compatible to their mammary counterparts. Most tumors seem to originate from intraductal lesions. Inverse cell polarity appears to facilitate progression of changes.

384 Immunohistochemical Expression of Survivin in Sebaceous Lesions

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Background: Survivin is a member of the inhibitor of apoptosis protein (IAP) family that has been implicated in both apoptosis inhibition and cell cycle control. This protein has recently attracted great interest as potential application in tumor therapy because its expression is amongst the most tumor-specific of all gene products. The pattern of expression of this protein has not been studied in sebaceous lesions.

Design: Four biopsy specimens diagnosed as sebaceous hyperplasia, four biopsy specimens diagnosed as sebaceous adenoma and two biopsy specimens diagnosed as sebaceous carcinoma were immunohistochemically studied for survivin (Santa Cruz, California, USA) expression in the sebaceous cells. The results were evaluated by a dermatopathologist, as positive cells/100 sebaceous cells, with no overlapping fields.

Results: The sebaceous hyperplasia lesions showed the lowest expression of survivin (average 2 positive cells/100 hyperplastic cells). There was intermediate expression in sebaceous adenoma lesions (average 8.2 positive cells/100 adenomatous cells). Sebaceous carcinoma lesions displayed the highest expression of surviving (average 12.45 positive cells/100 carcinoma cells).

Conclusions: The sebaceous lesions displayed increasing intensity of staining with progression from benign hyperplastic lesions to malignant lesions. With the observed immunophenotypic differences, these results support the theory of decreased apoptosis with lesions progressing from simple hyperplasia to carcinoma. These results may have some bearing on the pathogenesis of theses lesions.

385 Identification of Amplified Clonal T-Cell Populations in Skin Biopsies from Patients with Graft Versus Host Disease

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Background: Graft versus host disease (GVHD) is a common occurrence in patients after bone marrow transplantation (BMT). Its diagnosis can be difficult, especially in patients who present with diffuse erythroderma, which shows overlapping clinical and histologic features with other diseases, such as drug reactions or cutaneous T-cell lymphoma. We have seen rare cases of erythroderma after BMT, in which mycosis fungoides entered the differential diagnosis based on the histologic findings of a lichenoid inflammatory process with atypical lymphocytes. To explore the potential diagnostic value of clonality studies in this clinical setting, we studied of T-cell receptor gene rearrangements in tissues of patients with bona-fide GVHD.

Design: Skin biopsies from eleven patients with graft versus host disease were examined histologically, immunohistochemically and by molecular studies. TCR gamma PCR was performed using a commercial kit (InVivoScribe, San Diego, CA).

Results: The patients' age ranged from 6 years to 64 years. Six patients received BMT for acute leukemia (5ALL, one AML), three for lymphoma, one for chronic myelogenous leukemia, and one for renal cell carcinoma. Three patients developed erythroderma. A positive T-cell clone was found in three patients. Two of them had erythroderma and one did not. In patients with erythroderma, multiple biopsies revealed the same T-cell clone at different sites and different times in a given patient.

Conclusions: Some patients with GVHD, especially those with erthrodermic GVHD, may have a limited repertoire of T-cells that are clonally expanded and lead to positive results by PCR for TCR gene rearranegment. These findings further emphasize and expand known limitations and pitfalls of using molecular studies in the work-up of lymphomatoid cutaneous reactions.

386 D2-40 Is Highly Expressed in Primary Skin Adnexal Carcinomas but Negative in Adenocarcinoma Metastases to Skin

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Background: The monoclonal antibody D2-40 is a useful lymphatic endothelium marker with variable expression in epithelium. We evaluated D2-40 immunoreactivity in a series of primary skin adnexal tumors and adenocarcinoma metastases to skin, as well as CD15 expression in some of these tumors.

Design: The series has total of 91 cases including 10 sebaceous carcinomas, 10 squamous carcinomas, 4 porocarcinomas, 4 skin adnexal carcinoma, 4 trichilemmal carcinoma, 14 sebaceous adenomas, 15 syringoma , 9 hidradenoma, 7 eccrine poroma, 1 spiradenoma and 14 adenocarcinoma metastases to skin, including metastases from breast (6), lung (1), biliary tract (1), ovary (1), pancreas (1), colon (3) and thyroid (1). The monoclonal antibodies D2-40 and CD15 were used on paraffin sections with heat -induced epitope retrieval and Envision +HRP on a DAKO autostainer. The immunoreactivity of D2-40 and CD15 immunoreactivity was semiquantitatively evaluated.

Results: Based on intensity and extent of immunoreactivity of the tumor cells, the reactivity is categorized as follows:

	D2-40 Immunoreactivity	
Diagnosis (# of cases)	Diffuse Positive	Focally Positive
Sebaceous carcinoma (10)	60%	40%
Squamous carcinoma (10)	80%	20%
Porocarcinoma (4)	25%	75%
Trichilemmal carcinoma (4)	0%	100%
Other Skin adnexal carcinoma (3)	33%	67%
Benign primary adnexal tumor (46)	26%	57%
Cutaneous metastases (14)	0%	0%

	CD15 Immunoreactivity	
Diagnosis (# of cases)	Diffuse Positive	Focally Positive
Sebaceous carcinoma (8)	12.5%	62.5%
Squamous carcinoma (10)	30%	20%
Porocarcinoma (4)	75%	25%
Sebaceous adenoma (12)	0%	33.3%

Conclusions: 1.) All of the primary skin adnexal carcinomas and majority of the benign skin adnexal tumors showed variable D2-40 reactivity, but none of the cutaneous adenocarcinoma metastases was positive. The results are consistent with our prior observation that primary carcinomas from lung and breast are negative for D2-40. Therefore, D2-40 immunoreactivity can be used effectively to differentiate primary skin adnexal carcinomas from adenocarcinoma metastases to skin. 2) Although expressed in approximately 50% of the primary skin adnexal tumors, CD15 immunoreactivity did not seem to be specific for any skin tumor. The diagnostic utility of CD15 in differentiating those skin tumors is limited.

387 Spectral Imaging Microscopy Detects Lymphocyte Activation in Early Mycosis Fungoides

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Background: In early mycosis fungoides (MF), lymphocytes are usually small and abnormalities of the nuclei are difficult to appreciate in routine sections. Spectral Imaging Microscopy (SIM) can qualitatively and quantitatively assist in their evaluation.

Design: SIM employs image-enabled spectroscopic analysis with a standard light microscope. We used a prototype of a system (Nuance™, CRI, Inc.) that electro-optically selects narrow bands of light to be collected by a cooled, monochrome CCD camera. Images were taken every 10 nm from 420 nm to 720 nm. Data analysis software (Nuance™ Analyze, CRI, Inc.) yields both standard color (RGB) and spectral information at each pixel of the images.

Results: Spectra were studied from 40X fields from routine H&E slides of 104 biopsies of MF. Optical densities and spectral properties of small lymphocytes were determined and the hyperchromatic cells were pseudocolored to show their distribution.

Conclusions: In addition to the important architectural features of MF, SIM can define whether lymphocytes are hyperchromatic or normochromatic by setting appropriate threshold values. Spectral curves reveal increased eosinophilia in the nucleus that is similar to the signal from a nucleolus and/or nuclear indentations. Since lymphoblasts have enlarged nuclei with dispersed chromatin, a combination of spectral and spatial data is required for their analysis.

388 Myopericytoma of Skin and Soft Tissues: Clinicopathologic and Immunohistochemical Study of 52 Cases

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Background: Perivascular tumors comprise traditionally glomus tumor and hemangiopericytoma (HPC). Whereas glomus tumor represents a well-defined entity, the existence of HPC as an entity has been questioned, since many neoplasms are characterized by a HPC-like vascular pattern. Myopericytoma represents a recently delineated entity showing a HPC-like vascular pattern. A large series of myopericytoma of skin and soft tissues has been analysed to better characterize the clinicopathologic spectrum.

Design: 52 cases of myopericytoma of skin and soft tissues were retrieved and the histology reviewed. Immunohistochemical stainings using ASMA, desmin, and h-caldesmon antibodies were performed. Clinical data and follow-up informations were obtained from referring pathologists.

Results: 32 patients were male, and 18 were female (gender was unknown in 2 cases). Patient's ages ranged from 13 to 87 years (median:52 years). The lower extremities were most commonly affected (24) followed by the upper extremities (16), the head/neck region (5), and the trunk (2); exact location was unknown in 5 cases. 19 neoplasms were purely dermal, in 6 cases an extension into the subcutis was seen, and 23 as well as 4 cases arose in subcutaneous and deep soft tissues. Two cases were multicentric. Numerous thin-walled vessels and a concentric, perivascular arrangement of ovoid to round, myoid tumor cells was seen in all cases. However, a broad morphologic spectrum ranging from fibroma-like (3), myofibroma-like (2), angioleiomyoma-like (12), and HPC-like (13) neoplasms to classic myopericytomas (14) and immature, cellular lesions (2) was noted. In addition 4 intravascular and 2 malignant myopericytomas were found. Prominent cytologic atypia and increased proliferative activity was noted in 5 and 3 cases respectively. Immunohistochemically, all cases tested stained positively for ASMA, and 17 out of 21 cases for h-caldesmon, whereas desmin was only focally positive in 2 out of 31 cases. Despite marginal excision in 15 cases only 3 neoplasms recurred locally within 1 to 6 years.

Conclusions: Despite overlapping morphologic features to angioleiomyoma and myofibroma myopericytoma represents a distinct perivascular, myoid neoplasm of skin and soft tissues, characterized by a broad morphologic spectrum of concentrically, perivascularly growing myoid tumor cells that stain positively for ASMA and often for h-caldesmon.

389 High Levels of MMP-7, TIMP3 and VEGF Correlate with Aggressive Behavior in Merkel Cell Carcinoma

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Background: Merkel cell carcinoma (MCC) is a rare aggressive tumor that appears in sun-damaged skin in elderly people. MCC local aggressiveness is in relation to its capacity for extracellular matrix penetration, while MCC metastatic capability is facilitated by angiogenesis.

Design: We studied the expression of a panel of metalloproteases (MMPs), their tissue inhibitors (TIMPs), vascular endothelial growth factor (VEGF) and its receptor (FLK) and p53 in a series of 34 MCCs with known follow-up in order to define prognostic factors. Formalin-fixed, paraffin-embedded tissue from these 34 MCCs (31 primary skin tumors and 3 lymph node metastases) was used to construct a tissue microarray block. Sections were immunostained for MMPs (-1, 2, 3, 7, 9, 10-2, 10-5, 13, 14, 15 and 16), TIMPs (-1, 2, 3 and 4), VEGF, FLK and p53 using the avidin-biotin-peroxidase method. Expression intensity was semiquantitatively evaluated according to 4 grades (0=negative, 1=low, 2=medium and 3=high expression). Fifteen tumors behaved in a nonaggressive fashion, whereas 16 recurred locally or developed lymp node or systemic metastases.

Results: Aggressive MCCs demonstrated a statistically significant association with overexpression of MMP-7, TIMP3 and VEGF (p=0.037, 0.022 and 0.022, respectively). When expression grades were grouped in a binary fashion (0+1=negative vs. 2+3=positive), aggressive behavior showed a significant association with MMP-7

overexpression (p=0.011) and a trend to significant association with both p53 expression (p=0.053) and lack of FLK expression (p=0.080).
Conclusions: High expression levels of MMP-7, TIMP3 and VEGF in MCC are associated with aggressive behavior and could constitute useful prognostic indicators.

390 Clinicopathologic Significance of Dysadherin Expression in Cutaneous Malignant Melanoma: Immunohistochemical Analysis of 115 Cases

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Background: The E-cadherin-mediated cell adhesion system is frequently inactivated by multiple mechanisms and is involved in tumor progression in many types of cancer. Recently we have reported a novel cell membrane glycoprotein, dysadherin, which has an anti-cell-cell adhesion function and down-regulates E-cadherin.
Design: Expression of both dysadherin and E-cadherin was investigated immunohistochemically in 115 patients with cutaneous malignant melanoma to determine the correlation between the two molecules and their associations with both patient survival and the clinicopathologic features of the tumors.
Results: Dysadherin and E-cadherin were expressed at the cell membranes of melanoma cells. Fifty-two per cent of the tumors showed dysadherin immunopositivity, and 91% of the tumors showed reduced E-cadherin immunopositivity. There was no significant inverse correlation between dysadherin expression and E-cadherin expression. Increased dysadherin expression was significantly correlated with nodular subtype (p=0.042), Clark level (p<0.001), tumor thickness (p<0.001), lymph node metastasis (p<0.001), high TNM stage (p<0.001) and poor patient survival (p<0.001). Multivariate analysis of the patients' survival revealed that increased dysadherin expression was a significant predictor of poor survival (p=0.001).
Conclusions: Thus, increased expression of dysadherin is a significant indicator of poor prognosis in patients with cutaneous malignant melanoma.

391 Effect of Gleevec® (STI-571) on Melanoma Tissue

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Background: Protein tyrosyl phosphorylation is essential in intracellular signaling, mediating cell proliferation, survival, death, differentiation, migration, and attachment. Gleevec® (STI-571), an inhibitor of some tyrosine-kinase receptors (TKR), is currently being investigated as possible treatment in patients with metastatic melanoma (we have previously reported partial response lasting more than 1 year in 1 of 21 patients evaluated). This study analyzes the expression of TKR in melanoma lesions before and after treatment with Gleevec.
Design: Twenty-one patients with stage III-IV melanoma were treated with STI-571. Specimens with metastatic melanoma were obtained before and 2 weeks after treatment. Immunohistochemical analysis was performed using commercially available antibodies to known Gleevec target molecules including: c-KIT, phospho c-KIT, abl, abl-related gene (ARG), PDGFr-alpha, PDGFr-beta, and phospho-AKT. A positive result required labeling above background in at least 25% of the cells. Corresponding peptides were used to try to abrogate the immunoreaction.
Results: Expression for all antibodies was located in the cytoplasm of the tumor cells, with focal enhancement of the cytoplasmic membrane. At least 80% of the lesions expressed one of four TKR. After treatment with STI-571, melanoma specimens showed a lesser degree of TRK expression, being statistically significant for phospho c –KIT, phospho AKT, and ARG.
Conclusions: The decrease in expression of these TRK suggests a selective destruction of STI-571-sensitive melanoma cells and supports further exploration of STI-571 as a possible treatment in patients with metastatic melanoma.

392 p63 Immunohistochemical Expression Is Helpful in Differentiating Merkel Cell Carcinoma from Histological Mimics

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Background: Merkel cell carcinoma (MCC) is an aggressive primary neuroendocrine carcinoma of the skin, with tumor spread to regional lymph nodes in the majority of cases and distant metastases and death in at least one-third of affected patients. Histological differentiation from other tumors such as basal cell carcinoma and benign or malignant adnexal tumors can be difficult. The p63 gene is a member of the p53 tumor suppressor family with transactivating, apoptosis-inducing properties and is also essential in epithelial development and morphogenesis. p63 expression has not been previously studied in MCC.
Design: Immunohistochemical staining on formalin-fixed, paraffin-embedded tissues with mouse monoclonal anti-p63**,** prediluted (NeoMarkers, Fremont, CA) was performed on 5 MCC, 3 basal cell carcinomas, 4 poromas and 9 trichoblastomas. The number of positive staining cells were enumerated per 100 tumor cells within multiple non-overlapping high power fields, averaged with similar cases and reported as a percentage of cells stained.
Results: All MCC were negative for p63 expression (0.8%). All basal cell carcinomas (99.8%), poromas (98.9%), trichoblastomas (99.1%), were positive for p63 expression. The students t-test between MCC and all of the other neoplasms was significantly different (p=0.0002).
Conclusions: MCC is immunophenotypically distinct from many adnexal neoplasms and basal cell carcinoma. p63 may be a useful marker for distinguishing between MCC and other cutaneous neoplasms including basal cell carcinoma, poroma and trichoblastoma. Loss of p63 expression may have a role in oncogenesis of Merkel cell carcinoma.

393 Difference of C-kit (CD117) Immunohistochemical Expression in Benign Langerhans Cells in the Skin and Langerhans Cell Histiocytosis

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Background: C-kit (CD117) is a proto-oncogene that encodes a transmembrane receptor with tyrosine kinase activity. Its expression in gastrointestinal stromal tumors, melanocytes and mast cells, among others, is well documented. To our knowledge the expression of CD117 has not been investigated in benign Langerhans cells (LC) accompanying eczema in the epidermis versus systemic Langerhans Cell Histiocytosis (LCH).
Design: Immunohistochemical staining of formalin-fixed, paraffin-embedded tissues with mouse monoclonal anti-CD117 antibody (prediluted, NeoMarkers, Fremont, CA) was performed on three cases of cutaneous and systemic LCH and compared with the pattern and intensity of expression in LC co-labeled with CD-1a immunostain associated with three cases of eczema. Mean labeling cytoplasmic intensity for the antibody was calculated on a three level scale by a dermatopathologist. One hundred lesional cells were scored in each case in consecutive non-overlapping high power fields. The results were averaged and statistically examined with the students t-test.
Results: LC within the epidermis of eczema demonstrated faint cytoplasmic staining with CD117 (mean labeling intensity = 0.21), while the cells comprising the LCH demonstrated much stronger staining (mean labeling intensity =1.56), p= 0.001.
Conclusions: CD117 may be a useful marker for distinguishing among the lesional cells of LCH and Langerhans cells that populate and occasionally confound distinction with an inflammatory dermatosis. Furthermore, alterations in CD-117 expression may have important pathogneic considerations in LCH.

394 How Many Negative Nodes Are Needed? The Poisson Paradigm and Sentinel Lymph Node Sampling in Malignant Melanoma

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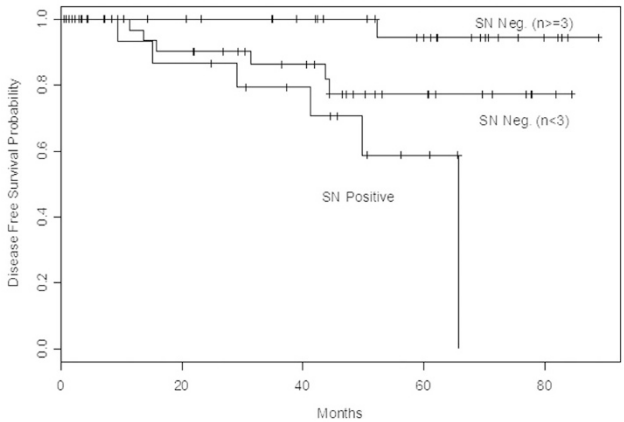
Background: The clinical staging of malignant melanoma necessitates the sampling of regional sentinel lymph nodes (SLN). On average, 2.1 SLN are removed per patient with 20% of patients having positive SLN. Of the 80% of patients with negative SLN, 11% exhibit metastatic recurrence despite complete excision of the primary lesion. Addressing one cause of false negative results, the undersampling of SLN, the Poisson paradigm suggests that the probability of finding positive SLN is directly related to the number of nodes sampled. Thus, in patients with negative SLN, the false negative rate should be highest when the number of nodes sampled is lowest. Using the Poisson model, Bayes' theorem suggests that the false negative rate should decrease significantly when three or greater SLN are sampled. Thus, we hypothesize that in patients with negative SLN, the subset of patients with only one or two nodes examined will have a shorter time to recurrence than will patients with three or greater nodes sampled.
Design: A group of 103 patients with completely excised malignant melanoma and concurrent SLN biopsy was subdivided into three groups: those with positive SLN (20), and those with negative SLN and either one or two nodes sampled (44) or three or more nodes sampled (39). Time to recurrence was measured over a median period of 41 months, and a log rank test was applied.
Results: A Kaplan Meier plot of disease free survival probability versus time illustrates that patients with negative SLN and fewer than three nodes sampled have a time to recurrence intermediate between those with negative SLN and three or more nodes sampled and those with positive SLN (p = .004).



Conclusions: These results indicate that in patients with malignant melanoma and negative SLN, the sampling of fewer than three SLN is related to a worse prognosis. Recognition of this trend should enable surgeons to more accurately gauge prognosis and plan therapy. Additionally, more aggressive intraoperative identification of SLN may result in more accurate staging of patients with malignant melanoma.

395 Melanomas with and without Contiguous Nevi Have Different Clinicopathologic Features, Risk Factor Profiles, and Patterns of Site Specificity

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Background: Melanoma has been hypothesized to arise by two different pathways: one associated with melanocyte proliferation and the other with chronic exposure to sunlight (J Natl Cancer Inst 2003;95:806-12). If this model is correct, then a reasonable corollary is that the risk factors associated with each pathway should differ. We analyzed melanoma associated with the presence or absence of a nevus, as a potential marker for the nevoid, or melanocyte proliferation pathway.

Design: Data was obtained from a population-based case-control study conducted in Connecticut in 1987-89 in which melanoma cases were identified through the Connecticut Tumor Registry (n = 650), and a series of age and sex-frequency matched controls were obtained by random digit dialing (n = 549) (Int J Cancer 1996;67:636-643). Phenotype, family history and sun exposure history in relation to melanoma characterized by presence or absence of nevus-association were analyzed using a case-control approach extended to two distinct events: melanoma with (MN+) or without (MN-) an associated nevus. Odds ratios and chi-square tests were used to measure associations.

Results: Our results show that MN+ melanomas were more often truncal, of the superficial spreading subtype, and thinner than MN- melanomas. The percentage of total melanomas that were MN+ peaked in the same age group (40-49 years) as did mean number of nevi. Having numerous nevi increased risk for MN+ more than MN- melanoma (P = .03). Having numerous back nevi conferred a site-specific risk for MN+ back melanoma (P = .002). Family history of melanoma showed a borderline differential association with MN+ melanoma (P = .05), while fair pigmentary traits and sun exposure history were not associated with differences in risk.

Conclusions: Our findings, that nevus-associated and de novo melanomas have different risk factors as well as different clinical and pathologic features, are compatible with the hypothesis that there may be two separate pathways to melanocyte transformation.

396 Fluorescence *In-Situ* Hybridization Confirmation of Cutaneous Acute Promyelocytic Leukemia

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Background: Cutaneous manifestations of acute promyelocytic leukemia (APL) are rare but well documented. Skin biopsies of APL can be difficult to confirm using morphology alone, and paraffin section immunophenotyping is not specific in separating APL from other acute myeloid leukemias involving the skin or inflammatory conditions with may mimic leukemia cutis. Fluorescence *in-situ* hybridization (FISH) has been shown to be a fast and effective method of detecting the PML/RARA gene fusion characteristic of APL in fresh blood and bone marrow samples. FISH has also been demonstrated to be effective in detecting other chromosomal rearrangements in paraffin embedded tissue (PET). This retrospective study of cutaneous lesions from patients with a history of APL evaluates the utility of performing PET FISH to confirm the presence of cutaneous manifestations of APL in formalin-fixed paraffin embedded skin biopsies.

Design: Three patients were identified with a history of APL and suspicious skin lesions that were biopsied. All patients had previous bone marrow findings of APL with characteristic morphologic findings, typical flow cytometry immunophenotype and cytogenetic studies which detailed the presence of the t(15;17) rearrangement. Nuclei were extracted from core biopsies performed on the formalin-fixed PET through a process of deparaffinization and enzyme digestion. FISH was performed using a dual color dual fusion PML/RARA probe. 200 nuclei were examined from each specimen.

Results: All cases showed evidence of the t(15;17) rearrangement by FISH, with 79%, 51%, and 16% positive signal patterns, each well above background limits. FISH performed on reactive tonsil (1000 total nuclei) yielded an average positive signal pattern of 0.3%. From this, a statistically significant background limit of 3.3% was determined for the PML/RARA probe (maximum range of positive signals for 200 nuclei plus two standard deviations). A skin-specific negative control was also used from a patient with lichenoid dermatitis which yielded a positive signal pattern of 0.5% (200 nuclei scored).

Conclusions: PET FISH appears to be a robust technique to detect cutaneous manifestations of APL in formalin-fixed paraffin-embedded skin biopsies.

Endocrine

397 Correlation between Genetic Alterations and Microscopic Features of Papillary Thyroid Carcinomas

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Background: It has been recently shown that papillary thyroid carcinomas can be initiated by one of three distinct molecular events: BRAF point mutations, RET/PTC rearrangements, or RAS point mutations. These alterations rarely overlap in one tumor and are found in at least two-thirds of all papillary carcinomas. In this study, we analyzed the association between these genetic events and microscopic features of papillary carcinoma as well as with their clinical and prognostic characteristics.

Design: Ninety-five papillary carcinomas were studied, of which 40 were positive for BRAF mutation, 16 for RET/PTC rearrangements, and 14 for RAS mutations. Histologic slides were evaluated in 64 cases and eight microscopic features were scored. This included six nuclear features: nuclear enlargement, irregularity of nuclear contours, chromatin clearing, nuclear crowding/overlapping, nuclear grooves, and nuclear pseudoinclusions. They were scored as 0, 1+ when present in < 10% of cells, 2+ when present in 10-50%, and 3+ when present in > 50% of tumor cells. Two 2 additional characteristics, tumor fibrosis and psammoma bodies, were also scored.

Results: At least 4 nuclear features were found in each tumor, with nuclear pseudoinclusions being the least frequent finding in all groups. BRAF mutations were more frequently associated with irregularity of nuclear contours and nuclear crowding, and with classic papillary growth and tall cell variant. RET/PTC had significant correlation with classic papillary morphology and psammoma bodies, whereas RAS mutations correlated with follicular variant and with lower frequency of psammoma bodies and tumor fibrosis. In addition, BRAF positive papillary carcinomas were associated with older age, higher frequency of extrathyroid extension, and more advanced tumor stage at presentation.

Conclusions: Papillary carcinomas harboring BRAF, RET/PTC, and RAS mutations all demonstrate at least 4 diagnostic nuclear features of papillary carcinoma. However, several significant variations in the frequency of nuclear features, psammoma bodies, and tumor fibrosis were observed between tumors harboring different mutations. In addition, significant correlation was found between BRAF mutations and tall cell variant, RET/PTC mutations and classic papillary carcinoma, and RAS mutations and follicular variant of papillary carcinoma. BRAF point mutations also showed significant association with features of more aggressive behavior of papillary carcinomas.

398 Allelic Imbalance of Tumor Suppressor Gene Loci in Benign and Malignant Lesions of the Thyroid in Patients Who Had Radiation as Children

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Background: Radiation in childhood is a known risk factor for thyroid carcinoma, but may also be related to benign nodular hyperplasias. Recent evidence indicates that radiation can induce clonal DNA damage at specific genetic loci in cell culture experiments that were analyzed by comparative genomic hybridization (CGH). In this study we analyzed lesions in thyroids from patients who had undergone radiation as children, patients with recent radiation for laryngeal carcinomas, and patients without radiation and with normal thyroids. We used a loss of heterozygosity analysis for the loci identified in the prior cell culture experiments.

Design: Thyroids from patients with a history of radiation, patients who had recent therapeutic external beam radiation for laryngeal carcinoma, and patients who had no radiation and underwent incidental thyroidectomy with laryngectomy for laryngeal carcinoma were included. Microdissection, DNA extraction, and PCR were performed for 18 different genetic loci defined by prior reported CGH studies of radiation damage in cell lines. A semiquantitative capillary electrophoresis analysis was used and loss of heterozygosity was considered present when the tumor allele ratio/normal allele ratio was <0.7 or >1.43. Frequency of allelic loss (FAL) was calculated from the number of losses/the number of informative loci.

Results: Forty cases of thyroids from patients with childhood radiation, 14 cases of recently radiated thyroids, and 15 cases of non-radiated thyroids were included. In the non-radiated and recently radiated thyroids, there were only extremely rare loci that had any evidence of allelic imbalance. In the thyroids from patients radiated as children, there were 10 cases with malignancy, 6 cases with adenomas, and 24 cases with nodular hyperplasia. Allelic losses were seen in carcinomas and in benign diseases at high frequency. Losses were seen at every locus with a range of 7 to 100% of the cases analyzed (mean 49.6%).

Conclusions: Radiation in childhood was associated with both benign nodular disease and carcinomas of the thyroid. The frequency of allelic imbalance was very high in all lesions in these patients, as compared to recently radiated and normal non-radiated thyroid glands. These data from human subjects support prior cell culture experiments detailing the genetic loci that are affected in radiation induced DNA damage in the thyroid and show that radiation induces genetic mutational damage even in benign proliferative processes in these thyroids.

399 Utility of Thyroglobulin Measurement in Fine-Needle Aspiration Biopsy Specimens of Lymph Nodes in the Diagnosis of Recurrent Thyroid Carcinoma

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Background: The most common site for the recurrence of papillary carcinoma of thyroid (PTC) is in regional lymph nodes. Ultrasound (US) imaging may identify abnormal appearing lymph nodes, suspicious for PTC recurrence. Although fine needle aspiration biopsy (FNAB) of abnormal lymph nodes is often diagnostic of recurrence, small (< 10 mm) or cystic lymph nodes may be non-diagnostic due to small numbers of tumor cells, and/or fibrosis in the tumor (either spontaneous or secondary to therapy). The measurement of thyroglobulin (TG) levels in FNAB specimens from lymph nodes suspicious for recurrent PTC can serve as an adjunct to the cytologic diagnosis.

Design: Nineteen abnormal appearing lymph nodes were aspirated under ultrasound guidance in 17 patients with a diagnosis of PTC. In addition to obtaining material for cytologic interpretation, an additional aspirate was obtained by FNAB and rinsed in 1 ml of normal saline for TG level measurements.

Results: The cytologic diagnosis included: reactive lymph node (RLN) 5, PTC 5 and inadequate for evaluation due to lack of epithelial cells (IE) 9 cases. TG levels were markedly elevated (539-17197250 ng/ml; normal <10 ng/ml) in all 5 patients with