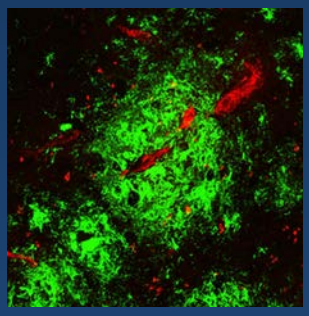
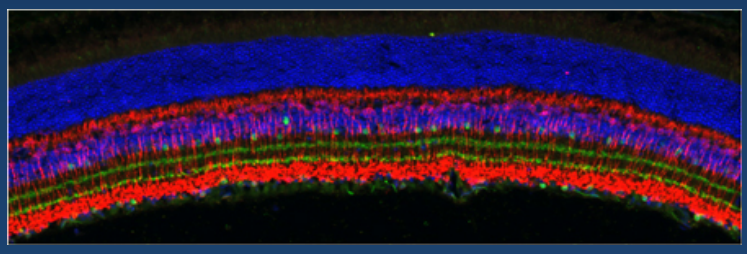
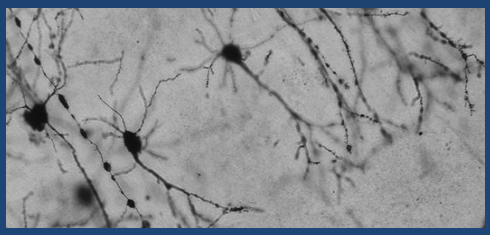
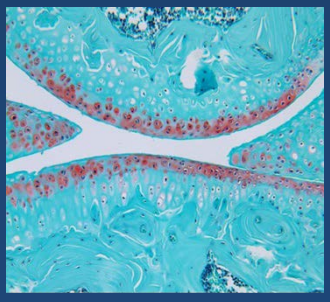
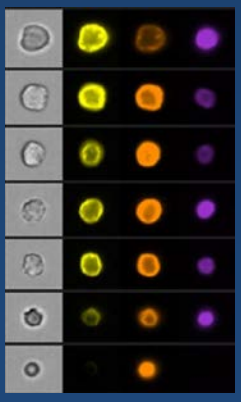
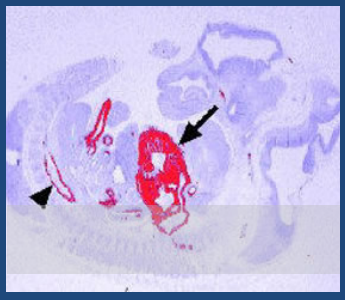
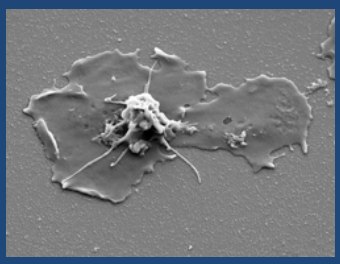
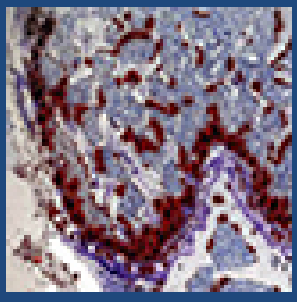
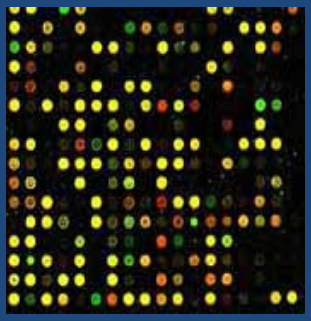
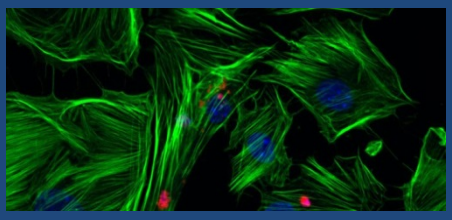
UNIVERSITY of ROCHESTER MEDICAL CENTER

Department of Pathology and Laboratory Medicine
36th Annual Research Day
June 13, 2016

2016 Pathology Research Day

Keynote Lecture

Scott Rodig, MD, PhD

Novel Tissue-based Biomarkers to Guide Immunotherapy

Dr. Rodig is a practicing hematopathologist at Brigham and Women's Hospital, an Associate Pathologist with the Specialized Histopathology Core Laboratory at the Dana-Farber/ Harvard Cancer Center, and an Associate Professor of Pathology at Harvard Medical School.

Dr Rodig received his MD and PhD (Immunology) at Washington University in St. Louis.

Dr. Rodig's research efforts are focused on the discovery, characterization, development, and application of novel molecular determinants of hematologic disease.

These investigational efforts involve the development of novel immunohistochemical markers that capture the activity of essential oncogenic signaling pathways in cancer using primary tissue samples. Most recently these efforts have been directed towards the characterization of the quality and quantity of immune responses within the tumor microenvironment to better guide immunotherapy.

Schedule of Events

Poster session in Flaum Atrium
All presentations in Class of '62 Auditorium

8:00 ~ 8:30 **Continental Breakfast Flaum Atrium**

8:30 ~ 9:30 **Welcome: Class of '62 Auditorium**
Dr. Bruce Smoller, MD, Chair, Department of Pathology and Laboratory Medicine

Oral Presentations
Mary M. Barrett, MD, GU Pathology Fellow
An Immunohistochemical Study of the Molecular Subtypes of Combined Urinary Bladder, Urothelial and Small Cell Carcinoma.

Shana Straub, MD, Resident, PGY 3
PLZF: A Sensitive and Specific Biomarker for Yolk Sac Tumor

Christopher W. Farnsworth, MS, PhD Class 2012
S. aureus Adaptation in Obesity and T2D in Orthopaedic Infection is Mediated by ClfA

Richard D. Bell, PhD Class 2014
Early Onset Morbidity and Mortality in Female TNF-Tg Mice with Inflammatory-Erosive Arthritis and Interstitial Pulmonary Disease

Eric Schott, MS, PhD Class 2013
Manipulation of the Gut Microbiome: A Potential Therapeutic Effect on Osteoarthritis in Obese Mice.

9:30 ~ 10:00 **Break ~ Poster Review ~ Breakfast Continued**

10:00 ~ 11:00 **Oral Presentations**

Robert Hoff, MS, PhD Class 2012
Progressive Dysfunction of Nrf2 Signaling with Age

Min Tian, MS, PhD Class 2010
Drosophila Cnc proteins regulates both antioxidant response and endoplasmic reticulum stress

Diana Agostini-Vulaj, DO, Chief Resident PGY 3
Distinction Between Telangiectatic/Inflammatory Adenoma and Mass Effect on Liver Sampling

Laura E. Bratton, MD, GI Pathology Fellow
IMP3 is Similarly Expressed Immunohistochemically in Intrahepatic and Extrahepatic Cholangiocarcinomas

Sohaib Abu-Farsakh, MD, PGY 2
High Expression of the Leaky Protein Claudin-2 in Esophageal Carcinoma and Precancerous Lesions is Significantly Associated with the Bile Salt Receptors VDR and TGR5

11:00 ~ 1:30	Luncheon and Juried Poster Session
	Afternoon Session
1:30 – 2:30	Oral Presentations
	Irina Lerman, MS, MSTP PhD Class of 2014 The Role of Neutrophil Elastase and SERPINB1 in Prostate Cancer
	Bradley N. Mills, MS, PhD Class 2011 Tumor-suppressive Potential of Dual Specificity Phosphatase 1 in Glioblastoma Tumors
	Nisha Patel, DO, PGY2 Green Neutrophilic Inclusions: Prognostic Marker of Acute Liver Injury and Impending Death
	Chad Hudson, MD, PhD, PGY 4 AID-Generated IGH Glycosylation Sites but Not Somatic Hypermutation Rate Differentiate Marginal Zone Lymphoma and Follicular Lymphoma
	Sapna Patel, MD, Associate Chief Resident, PGY 3 Flow Cytometric Detection of CD10 Expression in Diffuse Large B-Cell Lymphoma Can Reduce the Need to Perform the Complete Hans Algorithm by Immunohistochemistry
2:30 ~ 2:45	Break
2:45~3:45	Student Oral Presentations
	Sachica Cheris MD, MBA, PGY 3 Expression Levels of PR and Ki-67 are the Major Contributors for the Discordance Between ERS-Magee and RS-ODX
	Chao Xue, MS, PhD Class 2013 Cyclophilin A (CypA) is a Pathogenic Mediator of Pulmonary Arterial Hypertension
	Pei-Lun Weng, MS, PhD Class 2011 Non-neuronal Lineage Derived from Ascl3-expressing Cells in Homeostasis and Regeneration of the Olfactory Epithelium
	Salvador Peña, MS, MSTP, PhD Class 2011 Hypoxia and the Mitochondrial Unfolded Protein Response
4:00 ~ 5:00	Keynote Address **Dr. Scott Rodig, MD, PhD** Novel Tissue-based Biomarkers to Guide Immunotherapy
5:00	Closing Remarks: **Dr. Bruce Smoller, MD, Chair, Department of Pathology and Laboratory Medicine**
6:00 pm	Research Day Recognition Dinner and Reception Ballroom 384 at City Grill 384 East Ave., Rochester, NY 14607

Poster 1

Sapna Patel, MD

Resident: Associate Chief, PGY 3

Decalcification of metastatic breast cancer to bone and HER2 FISH analysis.

Sapna Patel, M.D., Loralee McMahon, Jill Henry, David G. Hicks, M.D.

Department of Pathology and Laboratory Medicine, University of Rochester Medicine, Rochester, NY

Context: 80% of patients who develop metastatic breast cancer will experience a skeletal metastasis. National guidelines recommend biopsy and repeat biomarker testing for metastatic breast cancers to guide further treatment. HER2 analysis in bone biopsies is challenging, due to the detrimental impact of decalcification solutions on tissue.

Design: We routinely separate bone from blood clot for biopsies in suspected skeletal metastases to provide non-decalcified tissue for possible biomarker analysis. A review of bone biopsies from metastatic breast cancer was done and all cases found to be negative or equivocal by HER2 IHC were reviewed. Testing for HER-2 utilized FDA-approved tests (HercepTest® DAKO). FISH utilized FDA-approved test (HER-2 IQFISH pharmDxTM DAKO).

Results: Eight cases of metastatic breast cancer to bone were negative or equivocal by HER2 IHC. Subsequent FISH testing performed on non-decalcified blood clot with tumor revealed two of eight patients as being HER2 amplified (25%). One of eight cases was found to be inadequate for FISH testing (Table 1).

Conclusions: Biomarker testing in skeletal biopsies is challenging due to the detrimental effects of decalcification. Given the clinical importance of this testing in recurrent breast cancer, techniques that avoid decalcification are needed. Our data shows that routinely separating blood clot from bone to avoid decalcification may yield tumor tissue for biomarker analysis to aid subsequent treatment planning

Poster 2

Sapna Patel, MD

Resident: Associate Chief, PGY 3

Flow Cytometric Detection of CD10 Expression in Diffuse Large B-Cell Lymphoma Can Reduce the Need to Perform the Complete Hans Algorithm by Immunohistochemistry

Sapna Patel, M.D., Chad Hudson, M.D., Ph.D., Richard Burack, M.D., Ph.D.

Department of Pathology and Laboratory Medicine, University of Rochester Medicine, Rochester, NY

Background: The current subclassification of diffuse large B-cell lymphoma (DLBCL) is based on gene expression profiling (GEP), which subclassifies DLBCL into germinal center-type (GCB) and activated B-cell type (ABC). This subclassification is important because it is believed that the two subtypes derive from different cells of origin, with the ABC-type of DLBCL having a more aggressive disease course. The Hans' criteria subclassify DLBCL via immunohistochemistry (IHC), a methodology more amenable to clinical use than RNA-based GEP. The Hans' algorithm is based on CD10, MUM1, and Bcl-6 expression in DLBCL, with CD10 expression being the most important marker as CD10 expression indicates GCB regardless of the expression of MUM1 and BCL6. As flow cytometry (FCM) for CD10 is often routinely performed on tissue samples, a result obtained by FCM regarding CD10 could preempt the need to perform IHC-based classification. However, it is possible that because FCM is more sensitive than IHC, this could lead to falsely subclassifying DLBCL specimens as GCB since CD10 positivity in Hans' criteria is strictly defined by IHC positivity. We aimed to test the concordance of CD10 expression determination by FCM and IHC.

Design: A retrospective survey of DLBCL cases, in which CD10 expression was measured first by flow cytometry and then by IHC, was conducted. A total of 50 cases were identified in which CD10 was measured by both FCM and IHC. The concordance between CD10 positivity by IHC and FCM was studied.

Results: Thirty-eight of the cases showed CD10 positivity by FCM, and CD10 positivity was confirmed in all of the cases by IHC. Twelve cases were negative for CD10, and these cases were found to be negative by IHC as well. In all, these data indicate that FCM had a positive predictive value (PPV), sensitivity, specificity, and concordance rate of 100%.

Conclusion: Even though it is thought that FCM is more sensitive than IHC, CD10 expression as measured by flow cytometry and IHC had a 100% concordance. Thus, detection of CD10 expression or absence by flow cytometry can be used in lieu of studying CD10 by IHC for the purposes of cell of origin classification by Hans' criteria and CD10 positivity by FCM alleviates the need of performing IHC pertaining to the Hans' algorithm.

Poster 3

Sapna Patel, MD

Resident: Associate Chief, PGY 3

Clinicopathologic Findings In Gynecologic Proliferations of the Appendix

Sapna Patel, M.D, Raul Gonzalez, M.D.

Department of Pathology and Laboratory Medicine, University of Rochester Medicine, Rochester, NY

Background: Appendiceal endometriosis is uncommon but well-described, and its clinical and microscopic presentation can vary. Other appendiceal gynecologic proliferations, such as endosalpingiosis and decidual lesions, are less studied. We conducted a review of these lesions to determine how clinical and pathologic observations correlated.

Design: We identified 67 cases of appendiceal gynecologic proliferations with available slides. Clinical presentation was recorded when available, and histologic findings were tabulated and correlated with clinical data.

Results: The cases included conventional endometriosis (55), endosalpingiosis (6), and decidual lesions (6). The endometriosis patients were 36 years old on average and presented with known endometriosis (23/47), acute appendicitis (11/47), ovarian/pelvic mass (7/47), intestinal mass (2/47), and other complaint (4/47). Only one patient who presented with appendicitis was ever diagnosed with extra-appendiceal endometriosis. All 55 cases demonstrated glands microscopically, 43 (78%) showed endometrial stroma, and 23 (42%) had hemosiderin. Disease was confined to the appendix tip in 22 cases (40%); none involved the appendix diffusely or reached the lumen. Stroma, hemosiderin, and tip confinement were equally prevalent in patients presenting with acute appendicitis and with known endometriosis. Only one case progressed to endometrioid adenocarcinoma. The endosalpingiosis patients were 42 years old on average and presented with acute appendicitis (2), pelvic mass (2), and other complaint (2). Endosalpingiosis was an incidental finding in all cases, confined to the serosa in 5 and extending intramurally in 1. The decidual cases included florid decidualized endometriosis (3), deciduosis (2), and pseudodeciduosis (1). Two decidualized endometriosis cases presented as vague acute appendicitis in pregnant patients, diffusely involved the appendix, and obliterated the lumen (image); the third arose in a nulligravid woman with endometriosis and involved the serosa and outer wall. The deciduosis and pseudodeciduosis cases were serosa-confined.

Results: Conventional appendiceal endometriosis can have several clinical presentations. Patients with it who present with acute appendicitis rarely develop it elsewhere. Otherwise, microscopic appearance and extent do not appear to correlate with symptomatology. Appendiceal endosalpingiosis is rare and effectively incidental. Decidualized endometriosis may overtake the entire appendix (unlike conventional endometriosis), whereas deciduosis and pseudodeciduosis are serosal lesions.

Poster 4

Sachica Cheris, MD, MBA

Resident: PGY 3

Expression levels of PR and Ki-67 are the major contributors for the discordance between ERS-Magee and RS-ODX

Sachica Cheris MD, MBA, Jianmin Wang MD, PhD*; Bradley Turner MD, MPH, MHA; Kristin Skinner MD; David G. Hicks MD; Ping Tang MD, PhD

Departments of Pathology and Surgical Oncology, University of Rochester Medical Center, Rochester, NY
*RTI Health Solutions, Research Triangle Park, NC

Recurrence scores of OncotypeDX (RS-ODX) have been routinely used in aid of clinical management for estrogen receptor (ER) positive, lymph node negative breast cancer (BC). Using several key pathologic parameters such as tumor grade, tumor size, and status of ER, progesterone receptor (PR), HER2, Ki-67, Klein et al developed three equations that produced Estimated Recurrence Scores (ERS-Magee equations) with relatively high concordance with the RS-ODX. The aim of this study was to identify factors that contribute to discordance between RS-ODX and ERS-Magee.

496 consecutive ER positive and HER2/lymph node negative BC cases with both RS and ERS between 2008 and 2015 were studied. Clinical and pathologic features such as patient age, tumor size, histologic grade (HG) (tubular formation [TF], nuclear grade [NG] and mitotic rate), and expression of ER, PR and Ki67 were documented. We documented the risk categories (low=0-18, intermediate=19-30 or high=31-100) of both methods for each patient. We defined a ≥ 10 point difference between the two methods as significantly discordant. Pathological features typically associated with better prognosis such as HG of 1, NG of 1, TF of 1, Mitotic activity of 1, ER>270, PR>270, Ki67<15%, and small tumor size were compared.

70% (346 cases) exhibit complete agreement between RS-ODX and ERS-Magee.30% of discordant cases were within one risk category away from each other. (1) 66/284 cases of low RS-ODX upgraded to Intermediate ERS-Magee; (2) 60/173 of Intermediate RS-ODX downgraded to a low ERS-Magee; (3) 23/39 high RS-ODX downgraded to intermediate ERS-Magee (Table 1). We distinguished all discordant cases from significantly discordant cases, and found that levels of PR expression were the most important pathologic factor that likely drives the shift between the low and intermediate RS and ERS; and the proliferative indices (mitotic count and/or Ki67) mostly impacted the downgrade from high RS-ODX to intermediate ERS (Table 2; Figure).

Expression of PR and measures of proliferation such as Ki67 and mitotic count appear to be important in discordance. Further studies are underway to investigate the details including clinical follow-up of each discordant case.

Poster 5

Sachica Cheris, MD, MBA

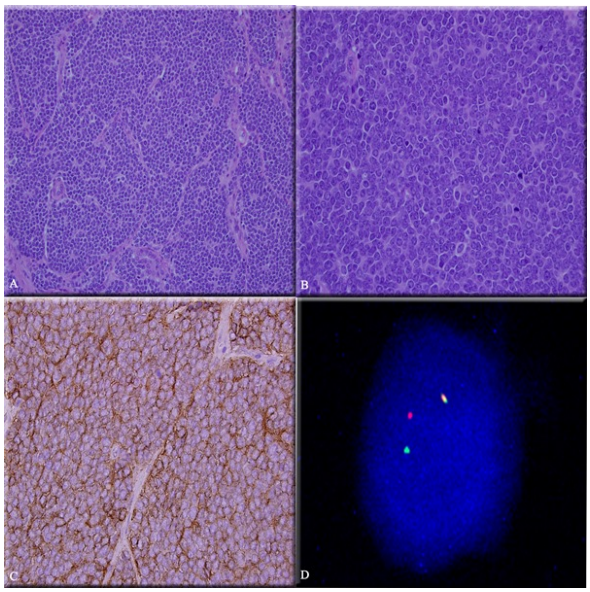
Resident: PGY 3

Primary Renal Ewing's Sarcoma/Primitive Neuroectodermal Tumor in a 65 Year Old Patient with a History of Bilateral Breast Cancer

Sachica Cheris, MD, MBA; Asif Shahab, MD; Faqian Li, MD, PhD.

University of Rochester Medical Center, Department of Pathology and Laboratory Medicine, Rochester, NY

Primary renal Ewing's sarcoma/primitive neuroectodermal tumor (ES/PNET) is a rare tumor characterized by genetic translocation t(11;22)and is typically seen in young adults. It's often difficult to diagnose in old patients, especially when there is a history of malignancy. We describe a case of primary renal ES/PNET in a 65-year old woman with treated bilateral breast cancer. On a computed tomographic scan, a heterogeneous mass was discovered incidentally in the upper pole of the kidney. Grossly, the tumor was unifocal, 11 × 8 × 4cm, white to pink with soft and fleshy cut surfaces. Microscopically, it consisted of sheets and large irregular nests of uniform small blue round cells with high nuclear to cytoplasmic ratios. No geographic necrosis was identified and the tumor was mitotically active (Figure 1A-B). No significant glycogen was identified with PAS and PAS-D stains. Immunohistochemical stains revealed that the tumor cells were diffusely positive for CD99 (Figure 1C) and focally positive for CD56 and synaptophysin. Rare stromal, but no tumor cells were positive for S100. No expression of cytokeratin AE1/AE3, cytokeratin 5, cytokeratin 7, cytokeratin 20, cytokeratin CAM 5.2, chromogranin, myogenin, p63, GATA3, TTF-1, PAX8, desmin, WT1, CD3, CD20, CD45 and CD138 was identified. Kappa and Lambda by chromogenic in situ hybridization were also negative. The morphological features and immunostaining results of this tumor were most consistent with ET/PNET. The diagnosis was further confirmed by fluorescence in situ hybridization which identified the presence of a EWSR gene rearrangement (Figure 1D).



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Poster 6

Chad Hudson, MD, PhD

Resident: PGY 4

Correlation Between Isotype Switch and Somatic Hypermutation in Follicular Lymphoma

Chad Hudson MD, PhD, Janice M Spence PhD, **Sapna S Patel MD** and Richard Burack MD, PhD.

University of Rochester Medical Center, Pathology, Rochester, NY, United States.

Background: B cell development is substantially shaped by the enzyme Activation Induced Cytidine Deaminase (AID) which both produces mutations in the immunoglobulin genes (somatic hypermutation (SHM)) and causes class switch from IgM to IgG/IgA expression. AID expression is induced in germinal center B cells and as such is highly expressed in follicular lymphoma (FL), a neoplasm of germinal center B cells. Follicular lymphomas display a range of evidence of AID-mediated effects, suggesting that measures of AID-mediated effects may serve as methods to further subclassify this lymphoma. Flow cytometric analysis of immunoglobulin heavy chain usage in FL is an intriguing candidate for such an assay. However, despite a general consensus that AID-mediated processes drive B cell maturation, there is little data addressing if the two known major effects of AID activity (point mutations and class switch) are actually correlated.

Design: Lymphoma cells from viable frozen samples obtained from 18 cases of follicular lymphoma were assayed for class switch via flow cytometry. Cells were stained with the following antibodies: anti-CD3, anti-CD19, anti-kappa, anti-lambda, anti-IgG, anti-IgM, and anti-IgA. The clonally rearranged IGH gene was amplified by PCR using *IGHV* family-specific with downstream *IGHJ* and *IGHC* primers and sequenced. Resulting sequences were analyzed using V-quest (IMGT.org) to determine *IGHV*gene usage, percent identity to germline sequences, and the presence or absence of large insertions/deletions/duplications (indels) within the downstream intronic portions of the IG gene.

Results: Of the 18 cases studied by flow cytometry, 11 expressed IgM and 7 expressed IgG or IgA, with class switch being confirmed at the DNA level. When grouped by switch status, there was significantly greater IGHV percent identity to germline in the IgM-expressing group compared to the IgG/IgA-expressing group (median: 90%, range: 82-91% vs. median: 86%, range: 72-88%, p=0.03). Class switch and the presence of downstream intronic indels trended together (p=0.1), and when the groups were categorized by both the presence/absence of indels and class switch, a significant difference in IGHV percent identity to germline was found between the indel and the no indel groups (p=0.004).

Conclusions: The two major activities of AID on the IGH locus, SHM and AID, are correlated in FL. These data suggest that a flow cytometric assay of heavy chain expression could be a surrogate marker for AID activity in FL. Whether this is a FL-specific or more generalizable correlation is under investigation.

Poster 7

Chad Hudson, MD, PhD

Resident: PGY 4

Elevated D-dimer Concentrations: Implications for Lupus Anticoagulant False Negatives

Chad A. Hudson, MD, Jonathan M. Soh, Grace W. Conley, Robert Miller,PhD, Michael Delski, Majed A. Refaai, MD

University of Rochester Medical Center, Pathology, Rochester, NY, United States.

We report a case of a 65-year-old male with a recent abdominal aorta repair in which post-operative recovery was complicated by a pelvic hematoma. PTT was found to be prolonged at 56.6 seconds (normal: 25.3-37.4). Further workup revealed a negative lupus anticoagulant (LA). However, the PTT mixing study was consistent with a non-specific anticoagulant inhibitor. Both factor VIII and IX activity were performed and showed decreased levels with indication of inhibitor interference. Additional testing showed a markedly elevated D-dimer concentration (28 mcg/mL; normal < 0.5). Reviewing the patient's medical records showed a prolonged PTT from four years earlier but no further workup. As it's extremely rare for a patient to have two specific factor inhibitors with no clinical findings, it was hypothesized that the LA result was falsely negative. At an outpatient follow-up visit 12 days later, the D-dimer was decreased to 6.32 mcg/mL and LA was found to be positive. Known causes of false negative LA tests include warfarin, heparins, factor deficiencies, and thrombosis. We hypothesized that high D-dimer concentrations interfere with the LA assay. To test this, we mixed known LA positive sera with sera known to have elevated D-dimer levels at a ratio of 1:1. Subsequent LA testing was negative in 75% of the cases (Table), suggesting that an elevated D-dimer level could lead to a false negative LA. To our knowledge, this is the first report indicating that high D-dimer concentrations may play an integral role in the confounding of LA testing.

Poster 8

Chad Hudson, MD, PhD

Resident: PGY 4

AID-Generated IGH Glycosylation Sites but Not Somatic Hypermutation Rate Differentiate Marginal Zone Lymphoma and Follicular Lymphoma

Chad Hudson MD, PhD, Hani Katerji MD, Janice M Spence PhD, Diana Adlowitz PhD and Richard Burack MD, PhD

University of Rochester Medical Center, Pathology, Rochester, NY, United States.

Background: B cell development is substantially shaped by the enzyme Activation Induced Cytidine Deaminase (AID) which both produces mutations in the immunoglobulin genes (somatic hypermutation (SHM)) and causes class switch from IgM to IgG or IgA expression. AID is induced in germinal center B cells and is highly expressed in follicular lymphoma (FL), a neoplasm of germinal center B cells. It has been shown that FL-derived IGH is more likely to have AID-generated glycosylation sites than IGH from non-neoplastic B cells and the malignant B cells in chronic lymphocytic leukemia, making glycosylation site status an intriguing marker for FL. Alternatively, marginal zone lymphoma (MZL) is a type of lymphoma in which AID expression varies widely and little is known about AID-generated IGH glycosylation. We compared clonal IGH sequences in FL and MZL specimens to determine if the level of SHM and the number of AID-generated glycosylation sites in IGH could be used to differentiate these lymphomas.

Design: The clonally rearranged IGH gene in 39 FL specimens and 30 MZL specimens was amplified by PCR using *IGHV* family-specific with junctional *IGHJ* region primers and sequenced. The resulting sequences were analyzed using V-quest (IMGT.org) to determine percent identity to germline sequences, a measure of SHM. AID-generated glycosylation sites were determined by analysis of the predicted protein sequence.

Results: Although there was significantly greater IGHV percent identity to germline in MZL compared to FL (median: 93%, range: 68-100% vs. median: 87%, range: 67-95%, $p<0.001$) there was overlap, with 4 of the MZL specimens having more SHM (lower percent identity) than the median for the FL specimens. Thirty-five of the 39 FL cases and 2 of 30 MZL cases had AID-generated IGH glycosylation sites ($p<0.001$). The 2 MZL cases had 1 glycosylation site, whereas the FL cases ranged from having 1-4 glycosylation sites, leading to a significant difference in the number of glycosylation sites ($p<0.001$). Amongst all specimens, there was a significant correlation between SHM and the number of glycosylation sites (Correlation Coefficient = -0.35, $p=0.003$). In sum, odds ratio analysis indicates that FL is 120 times more likely to have an AID-induced glycosylation site than MZL and this effect is not explained by the slight difference in the extent of SHM.

Conclusions: AID-generated glycosylation sites are a general feature of FL but not MZL despite the overlapping rates of SHM. AID-generated glycosylation status may be useful in differentiating between FL and MZL.

Poster 9

Fu-Ju Chou

Program Year: 2014

Advisor: Chawnshang Chang, PhD

Androgen receptor (AR) degradation enhancer ASC-J9® increases radiation sensitivity to better suppress prostate cancer progression

Fu-Ju Chou[1], Yin Sun[2], Yuhchyau Chen[2], Peter Keng[2] and Chawnshang Chang[1]

[1]George Whipple Lab for Cancer Research, Departments of Pathology, Urology, [2]Radiation Oncology and The Wilmot Cancer Center, University of Rochester Medical Center, Rochester, NY 14642, USA

While androgen-deprivation-therapy (ADT) and radiation therapy (RT) are currently used to treat locally advanced prostate cancer (PCa). Their mutual influence, however, remains unclear. Here we found that RT may result in unwanted side effect such as radiation cystitis in bladder. Besides RT also increase the protein expression of PCa androgen receptor (AR) protein that currently ADT or antiandrogen fails to suppress. AR level increase will transactivate AR downstream DNA damage response protein which make PCa much more resistant to radiation. Targeting AR with the AR degradation enhancer ASC-J9® resulted in increasing radiation sensitivity which is better to suppress PCa, and only cause very little effect on normal bladder cells. Mechanism dissection suggested that degradation of this radiation-increased AR by ASC-J9®, and not by anti-androgens including Casodox or Enzultamide, increased the radiation-induced apoptosis *via* suppressing the pATR-CHK1 signals and lead mitotic catastrophe. ASC-J9® could also increase radiation sensitivity *via* altering the Homologous recombination repair pathway as well as inducing DNA damage by ROS generation and altering GSH level. Together, these results suggest that combined ASC-J9® with ionizing radiation (IR) may represent a novel therapy to better suppress PCa progression

Poster 10

Ronghao Wang

Program Year: 2014

Advisor: Chawnshang Chang, PhD

Enzalutamide-induced a trans-splicing event of AR transcript in prostate cancer cell

Ronghao Wang[1], Yin Sun[1], Chawnshang Chang[1]

[1]George Whipple Lab for Cancer Research, Departments of Pathology, Urology, Radiation Oncology, and The Wilmot Cancer Center, University of Rochester Medical Center, Rochester, NY 14642, USA

Prostate cancer (PCa) is the second leading cause of cancer-associated death in western countries. Although ADT shows promising therapeutic effect to cure PCa, the castration resistance would invariably develop due to continuous activation of AR signaling. A more powerful anti-androgen enzalutamide (Enz, also called MDV3100) was recently approved to treat castration-resistant prostate cancer (CRPC) that can extend PCa patients' survival by 4.8-months. Interestingly, we unexpectedly found that a new AR variant (named as AR-v33 with a duplicated exon3) was dramatically induced in our established Enz-resistant PCa cells. Sequencing result also confirmed the existence of the exon3 duplication of AR in Enz-resistant cells, and AR-v33 frequently exists in CRPC tissues than benign prostate tissues. Importantly, our preliminary data demonstrated that the induction of this new AR-v33 variant is not due to genomic alteration, and probably, it may be generated from a trans-splicing event of pre-AR transcript. Future study will be focused on the biological functions of AR-v33 and its responsible signaling molecules.

Poster 11

Irina Lerman, MS

Program Year: 2014, MSTP

Advisor: Stephen Hammes, MD, PhD

The Role of Neutrophil Elastase and SERPINB1 in Prostate Cancer.

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Tissue infiltration and elevated peripheral circulation of myeloid derived granulocytes or neutrophils is associated with poor prostate cancer outcomes in several independent studies and meta-analyses. As such, we are interested in studying the role of neutrophil elastase (NE), a serine protease released upon immune cell activation, in prostate cancer development and progression. NE has pathogenic roles in several cancers, including breast, lung, and colon. Furthermore, the endogenous NE inhibitor SERPINB1 is down regulated in prostatic intraepithelial neoplasia and prostate cancer as compared to normal prostatic epithelium. Mechanisms responsible for the reduced expression of SERPINB1, as well as its functional significance, are unknown; yet these observations suggest that the balance between NE and its inhibitors may be physiologically important within the prostate. Here we report that NE is present in prostate cancer xenografts established in nude mice and is supplied by infiltrating cells, as determined by quantitative PCR and immunohistochemistry. Furthermore, NE activity abundantly localizes to xenografted tumors and can be visualized by an NE-specific optical probe *in vivo* and *ex vivo*. Importantly, treatment with sivelestat, a SERPINB1 pharmacomimetic and NE inhibitor, significantly decreases primary xenograft growth. Mechanistically, we find that NE stimulates extra-nuclear-signal-regulated (ERK) signaling in part by trans-activating the epidermal growth factor receptor (EGFR), activates ERK dependent transcription of the proliferative gene cFOS, and increases migration and Matrigel invasion *in vitro.* Immunohistochemistry on human prostate cancer biopsies reveals that NE is expressed within the stromal compartment and corpora amylacea, while SERPINB1 expression is mainly within the prostatic epithelium. Finally, examining large-scale gene expression profiles of human prostate cancer, we find that low SERPINB1 expression is indicative of higher-grade cancer and significantly worse prognosis. Studies are underway to further elucidate the potential tumor suppressor role of SERPINB1 and to investigate its relation to NE activity inhibition in prostate cancer.

Poster 12

Xiaoting Ma, MS

Program Year: 2013

Advisor: Stephen Hammes, MD, PhD

Paxillin regulates genomic programming in prostate cancer

Xiaoting Ma[1,2], Anindita Biswas[2], Stephen R. Hammes[1,2]

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Abstract: Paxillin, well known as a focal adhesion associated adaptor protein, is extensively involved in cellular signal transduction such as mediating cytoplasmic Erk1/2 signaling. However, recent studies have shown that paxillin also plays a critical role in nuclear Erk1/2 signaling, as well as in the regulation of both genomic and nongenomic steroid signaling. In prostate cancer cells, paxillin serves as a critical liaison between cytoplasmic and nuclear signaling, mediating not only androgen or growth factor induced extranuclear Erk1/2 signaling, but also Erk1/2 and Androgen Receptor (AR)-induced intranuclear gene transcription. In fact, paxillin expression is upregulated in human prostate cancer tumor microarrays, suggesting it as an important mediator of prostate cancer progression. Here, we use an RNA-Seq strategy to take a global look at the paxillin regulated transcriptome in prostate cancer cell lines. RNA-seq data from PC3 cells with reduced paxillin expression after siRNA treatment revealed that paxillin activates several pro-proliferative pathways, including the CyclinD1-Rb-E2F1 pathway. Paxillin also downregulates expression of genes involved in pro-apoptotic pathways, including CASP1 and TNFSF10. Similar gene regulation patterns were also observed in androgen responsive LNCaP cells. Additionally, knocking down paxillin in dihydrotestosterone (DHT) treated LNCaP cells eliminated around 1000 DHT regulated genes, some of which are well known in endocrine therapy resistance. Finally, a paxillin inhibitor, JP-153, exhibits inhibitory effects on cell proliferation and decreases expression of many of the aforementioned genes. Thus, in prostate cancer, paxillin appears to promote proliferation, inhibit apoptosis, enhance androgen responsive gene transcription, and promote hormone resistance. Paxillin might therefore potentially serve as a therapeutic target for both androgen-sensitive and castration resistance prostate cancer.

Poster 13

Chia-Hao Wu

Program Year: 2015

Ad Hoc Advisor: Robert Mooney, PhD

Bladder Cancer Extracellular Vesicles Induce Chronic ER Stress Trigger Tumorigenesis, A Novel Mechanism Of Field Effect

Chia-Hao Wu[2], Christopher Silvers[1], Edward Messing[1], Yi-Fen Lee[1,2]

University of Rochester Medical Center, Department of Urology[1], Department of Pathology[2]

The high incidence of tumor recurrence makes bladder cancer the most expensive cancer to treat over the lifetime of the patient. Therefore, there is a great need for understanding the underlying biology of these tumors in order to identify novel therapeutic targets. Field effect describes a preconditioned area with either normal histological findings carrying gene mutations or altered morphology. The precancerous field is an important factor for cancer recurrence , however the contributing factors for field effect are not clear. Extracellular vesicles (EVs) are a population of small membrane bound vesicles, size ranging from 30-100nm, secreted by most types of cells, and substantially increased secretion in cancer cell. EVs exhibit the molecular signature of the origin cell, including a wide variety of proteins, nucleic acids and lipids. Recent studies have sparked interest in how these vesicles can participate in intercellular communication during disease development and progression. Here we propose long-term exposure of cancer EVs could be a driving force for preconditioned urothelial cell transformation via increased chronic ER stress and inflammation induced by cancer EVs. SV-HUC cells were immortalized, however not transformed, by introducing SV40 large T antigen. The SV-HUC cells were exposed to high grade cancer(TCCSUP) EVs for 8 weeks and 13 weeks. Analysis of these cancer EVs exposed cells shows the tolerance for DNA damage as well as cell-cell contact inhibition increase in a time dependent manner. Interestingly, only the SV-HUC cells exposed to high grade cancer EVs for 13 weeks gain the function of invasion and growth of tumor in nude mice. Examination of these cells shows increase in ER stress signals and inflammation cytokines. As a conclusion, SV-HUC cells are transformed by long-term exposure to TCC-SUP EVs, and possibly caused by increased ER stress and inflammation.

Poster 14

Carlos J. Ortiz-Bonilla

Program Year: 2015

Ad Hoc Advisor: Robert Mooney, PhD

Extracellular vesicles as a possible mechanism for BCG immunotherapy in bladder cancer

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Bladder cancer (BC) is the most prevalent cancer of the urinary tract and represents the fourth most common malignant disease in males. Treatment for non-muscle invasive (NMI) BC includes trans-urethral resection of the tumor followed by Bacillus Calmette-Guérin (BCG) immunotherapy. Even though BCG immunotherapy is considered the most successful therapy to prevent recurrence and progression of NMI BC, its mechanism of action is still not well understood. As such, it hinders further improvement of applying BCG immunotherapy**.** Interestingly, unpublished data in our lab has shown that patients who respond to BCG have significantly increased extracellular vesicle (EV) numbers in their urine; in contrast, in BCG non-responders, their urine EV numbers remained low and unchanged throughout the treatment. It has also been reported that EVs can mediate cellular communication to activate the immune system. In this study, we want to explore the effect of BCG immunotherapy in the secretion of EVs by BC cells. We hypothesize that these EVs could mediate communication between BC and immune cells to activate the immune system. To test our hypothesis, we used two BCG-responsive BC cell lines: MB49 murine and T24 human cell lines. First, we treated both cell lines *in vitro* with BCG, isolated the secreted EVs, and characterized them using the Nanoparticle Tracking Analysis. Then, we isolated the mRNA and performed a quantitative PCR to examine the expression of immunological molecules. We also measured the protein expression changes in both cells and secreted EVs after BCG treatment using Western Blot analysis. The results show that BCG treatment increased the release of EVs by both MB49 and T24 cell lines. In addition, BCG treatment resulted in an upregulation of antigen presentation genes in both cell models. These results support our hypothesis, allowing us to continue exploring the role of extracellular vesicles in BCG immunotherapy.

Poster 15

Laura E. Bratton, MD

Fellow: GI Pathology

IMP3 is similarly expressed immunohistochemically in intrahepatic and extrahepatic cholangiocarcinomas

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Insulin-like growth factor-II (IGF-II) messenger RNA binding protein-3 (IMP3), also known as IMP-3, KOC, and L523S, is a 580 amino acid oncofetal RNA binding protein encoded by the IGF2BP3 gene. IMP3 has been demonstrated to be overexpressed in multiple human malignancies, including pancreatic adenocarcinoma and hepatocellular carcinoma. Limited studies have been performed on IMP3 expression in intrahepatic cholangiocarcinomas (ICCs). The aim of this study was to examine IMP3 expression in cholangiocarcinomas, including examination of ICC and extrahepatic cholangiocarcinomas (ECCs).

Forty three cholangiocarcinomas representing biopsy and resection specimens were immunohistochemically studied using a monoclonal antibody against IMP3. Cytoplasmic staining was considered positive. The percentage of positively stained tumor cells was recorded and the staining intensity was graded as weak, moderate, or strong. A p value of <0.05 was considered statistically significant.

Histologically, there were 23 ICCs and 20 ECCs. Immunohistochemically, 6 (14.0%) of the cholangiocarcinomas were negative for IMP3 staining, while 10 (23.3%) demonstrated staining in 5-50% of neoplastic cells, and 27 (63.0%) demonstrated staining in greater than 50% of neoplastic cells. Staining intensity paralleled percentage of positive cells, with weak staining intensity being demonstrated in 7 of the cases, all of which had between 5 and 40% of tumor cells staining. Moderate staining was observed in 8 cases, with 40 to 80% of tumor cells being positive in these cases. Strong staining was demonstrated in 22 cases, all of which had at least 80% of tumor cells staining with the IMP3 marker. When stratified by location, there was no significant difference in the staining profiles of ICCs and ECCs. Lymphovascular and perineural invasion were most commonly associated with ECCs with strong and diffuse IMP3 staining. When examining patients who died from their malignancy, IMP3-negative ICCs averaged 1.22 years of survival (0.57-1.59) as compared with 1.71 (0.3-4.58) years if IMP3-positive ICC. In comparison, IMP3-negative ECCs averaged 2.34 years of survival (2.26-2.42) as compared with 1.40 (0.07-3.98) years if IMP3-positive ECC.

Among the 43 examined specimens, IMP3 immunohistochemical staining patterns were similar in ICC and ECC, with the majority of tumors demonstrating strong and diffuse staining. Previous reports of IMP3 expression in ICCs have demonstrated that IMP3 expression is associated with poor outcome. This study lends support to the prior findings despite a longer average survival time for IMP3-positive ICCs as compared with IMP3-negative ICCs in this limited study. Additionally, our finding suggest that ECCs behave similarly to ICCs in regards to IMP3.

Poster 16

Meenal Sharma, MD

Resident: PGY 4

Combination of Astrocyte Elevated Gene 1 and Glypican 3 Immunochemistry Improves Diagnostic Accuracy of Hepatocellular Carcinoma

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Background: The expression of Glypican 3 (GPC-3) is often negative or patchy/focal positive in well to moderately differentiated hepatocellular carcinoma (HCC), though GPC-3 is a specific marker in differentiating poorly differentiated HCC from benign hepatocellular proliferation or metastatic carcinoma. Additional markers are needed for assisting pathological diagnosis of early hepatocellular neoplasms. Recently, astrocyte elevated gene 1 (AEG-1) was identified as a new diagnostic marker for HCC. We studied the diagnostic values of AEG1, GPC-3 and the combination of both markers in HCC.

Methods: Expressions of AEG-1 and GPC-3 were studied by immunohistochemistry in a total of 46 resection specimens from 37 HCC patients. They included 37 HCCs (9 well, 20 moderately and 8 poorly differentiated) with adjacent nonneoplastic liver tissues (NON), and 9 dysplastic nodules (DN). The staining of AEG-1 and GPC-3 were evaluated and assigned to one of the following categories: negative, weak staining or $\leq$5% of tumor cells with moderate to strong staining; positive, moderate or strong staining seen in >5% but $\leq$50% (focal), or diffuse, in >50% (diffuse) of the tumor cells. Fisher exact analysis was applied to compare the proportions between the groups.

Results: Positive AEG-1 and GPC-3 staining was seen in 91.9% (34/37) and 56.8% (21/37) of HCCs respectively. Diffuse AEG-1 staining was seen in 89% of HCCs, 5% of DNs and 11% of NONs. Diffuse positive GPC-3 was found in 41% of HCCs, 5% of DNs and 0% NONs. 14 GPC-3 negative (4 well, 6 moderately, and 4 poorly differentiated) and 5 focal positive (1 well, 4 moderately differentiated) HCCs showed diffuse staining of AEG-1. Two AEG-1 negative HCCs showed focal positive GPC-3 staining. One moderately differentiated HCC was negative for both AEG-1 and GPC-3. The sensitivity and specificity for diffuse AEG-1 staining alone was 89% and 95% respectively. The sensitivity and specificity for diffuse GPC-3 staining was 41% and 100% respectively. The sensitivity and specificity was 94% and 95% for the combination of diffuse AEG-1 and GPC-3 staining.

Conclusions: AEG-1 shows better sensitivity than GPC-3, whereas GPC-3 appears more specific for HCC. AEG-1 combined with GPC-3 may be a promising approach to discriminate HCC from benign hepatocellular proliferation.

Poster 17

Sohaib Abu-Farsakh, MD

Resident: PGY 2

High expression of the leaky protein Claudin-2 in esophageal carcinoma and precancerous lesions is significantly associated with the bile salt receptors VDR and TGR5

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Background: Claudins are a family of integral membrane proteins and are components of tight junctions (TJs). Many TJ proteins are known to tighten the cell structure and maintain a barrier. In contrast, Claudin-2 is a leaky protein that plays an opposing role and increases cell permeability. Recently, we found that VDR enhanced Claudin-2 expression in colon and that bile salt receptors VDR and TGR5 were highly expressed in esophageal adenocarcinoma (EAC) and precancerous lesions. Here, we examined the expression of Claudin-2 in EAC and precancerous lesions and its association with VDR and TGR5 expression.

Materials and methods: Claudin-2 expression was examined by immunohistochemistry on tissue microarrays, containing EAC, high grade dysplasia (HGD), low grade dysplasia (LGD), Barrett's esophagus (BE), columnar cell metaplasia (CM), squamous cell carcinoma (SCC), and squamous epithelium (SE) cases. Intensity (0 to 3) and percentage were scored for each case. High expression was defined as 2-3 intensity in ≥ 10% of cells.

Results: Claudin-2 was highly expressed in 77% EAC (86/111), 38% HGD (5/13), 61% LGD (17/28), 46% BE (18/39), 45% CM (29/65), 88% SCC (23/26), and 14% SE (11/76). It was significantly more highly-expressed in EAC, SCC and glandular lesions than in SE and more in EAC than in BE and CM. No significant difference in Claudin-2 expression was found between CM, BE, LGD, and HGD. A significant association was found between Claudin-2 expression and VDR and TGR5 expression. No significant association was found between expression of Claudin-2 and age, gender, grade, stage, or patients' survival time in EAC and SCC.

Conclusions: We conclude that Claudin-2 might play a novel role in the development and progression of esophageal mucosal metaplasia, dysplasia and carcinoma. Claudin-2 expression is significantly associated with VDR and TGR5 expression. The functional relationship between Claudin-2, VDR and TGR5 will be studied in future.

Poster 18

Sohaib Abu-Farsakh, MD

Resident: PGY 2

Development of anti-e after platelet concentrate transfusion

Sohaib Abu-Farsakh, Renee Bowen, Neil Blumberg, Majed A. Refaai

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Platelet concentrates (PC) is prepared from donated whole blood units by centrifugation and subsequent pooling of 4-6 units. Thus, PC may contain a small amount of donor RBC. We report a case of a 60 year-old male patient with past medical history of coronary artery disease, who presented to our emergency department with shortness of breath. Previous medical history indicated a recent admission, one month prior, to another local hospital for shock and respiratory failure. Type and screen (T/S) done at that time reveal an O+ blood type with negative antibody screen. During that admission, the patient received only 5 doses of PC (2-group O+, 2-group O-, and 1-group A-). He denied any other recent blood product transfusions at any other facility. Our current T/S, and before any transfusions, showed a positive antibody screen. A panel for antibody identification was performed. The antibody reacted with 9 out of 10 reagent cells. The reactivity was predominately at room temperature and 37^{O}C phases indicating an IgM class antibody. Some reactivity was also seen at AHG (anti-human globulin) phase indicating an IgG class antibody. The auto control was negative. Testing additional cells revealed anti-e specificity. Patient's "e" antigen typing was performed and found to be negative indicating that this is a true allo-antibody. Since anti-e antibody predominantly exists as an IgG immunoglobulin class antibody, the presence of IgM class reactivity suggests that this may be a newly developed antibody. This case illustrates that antibodies to RBC antigens can develop after platelet transfusion.

Poster 19

Mary Barrett, MD

Fellow: GU Pathology

An Immunohistochemical Study of the Molecular Subtypes of Combined Urinary Bladder Urothelial and Small Cell Carcinoma.

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Background:
Recent studies have shown that urinary bladder cancer can be classified into three molecular subtypes (luminal, p53-like and basal), which bear prognostic and therapeutic significance. Small cell carcinoma (SmCC) of the bladder is rare and frequently coexists with conventional urothelial carcinoma (UC). The immunohistochemical (IHC) profile of combined bladder UC and SmCC has not yet been studied. The aim of this investigation is to elucidate this IHC profile and compare the IHC of combined tumors to the IHC of the molecular subtypes of bladder cancer.

Design:
A total of 18 combined UCs and SmCCs were studied. The amount of SmCC in the tumor ranged from 5% to 95%. The UC was present as carcinoma in situ (CIS), invasive carcinoma, or both. Immunohistochemical markers used included GATA3, CK20, HER2, p53, Uroplakin III, CK5, CK14 and CD138. UC components were subclassified into luminal and basal types based on their IHC phenotypes as defined by previous molecular studies.

Results:
Among the UCs of the 18 cases, 12 (67%) were luminal-type (CK20+/GATA3+/HER2+), of which 10 (83%) had mutated p53 (Tp53), 2 (17%) had wild type p53 (Wp53), and 10 (83%) had moderate to strong HER2 expression. Four (22%) were basal-type UCs (CK5+/CK14+), of which 3 (75%) had Tp53 and 1 (25%) had Wp53. Two (11%) were Wp53 UCs and did not express other markers. The UC markers were mostly lost in the coexisting SmCCs (6 GATA3+, 2 CK20+, 0 HER2+, 2 CK5+, 0 CK14+), except for p53, in which the SmCC preserved the same pattern as the UC (13 Tp53 and 5 Wp53). None of the tumors expressed Uroplakin III, and CD138 was expressed in majority of the UCs (17/18, 94%) as well as SmCCs (11/18, 61%).

Conclusions:
SmCC was much more commonly associated with luminal type than basal type UC. SmCC also tended to lose the coexisting UC markers, except for p53. The 5 cases with wild type p53 may represent the p53-like bladder cancer subtype; however, further p53 gene expression study would be helpful for confirmation. Since the luminal type of bladder cancer is reported to have better prognosis and good response to cisplatin-based therapy as well as possible HER2 targeted treatment (due to its high expression of HER2), recognition of this association may be useful in guiding clinical management of these combined bladder cancers.

Poster 20

Nisha Patel, DO

Resident: PGY 2

Green Neutrophilic Inclusions: Prognostic marker of acute liver injury and impending death

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Background

Bright green inclusions in neutrophils have recently been described in critically ill patients and reported to strongly correlate with acute liver injury and high mortality rates. To date, less than thirty cases have been described in literature. Due to their low incidence, combined with their rarity within samples (1-2% of cells on peripheral blood smear) these inclusions remain poorly characterized. In addition, evidence on their biochemical properties has yet to be fully characterized. Elucidating the composition and clinical setting in which these inclusions emerge would allow us to develop more sensitive assays. Earlier detection methods could serve clinical utility as a prognostic marker for acute liver injury and impending death.

Methods

Peripheral blood smears (n=17) were prospectively collected over a 2 year period. Stains for Oil red O, Ziehl–Neelsen acid-fast, copper, iron, myeloperoxidase, periodic acid–Schiff–diastase (PAS-D), and bilirubin stains were conducted (n=3). Electron microscopy (n=3) was performed for ultrastructural characterization. Fluorescence microscopy and flow cytometry (n=2) using BODIPY, a lipophilic dye was used to analyze uptake within inclusions and to quantify lipid content in neutrophils compared to normal controls.

Results

A total of 17 patients were identified with median age of 53 years (range 17-73). Elevated liver enzymes were found in 16/17 (94%) patients at time of detection. Liver injury was multi-variable, ranging from acetaminophen toxicity, portal vein thrombosis, and septic shock. Twelve of the patients (70%) died; nine of whom died within a week of initial green inclusion detection. Inclusions were positive for Oil red O and Ziehl-Neelsen acid-fast. Electron microscopy of the inclusions showed osmiophilic (lipid-rich) content, validating and further supporting lipofuscin/ceroid like characteristics. Of note, unlike lipofuscin, the inclusions were not autofluorescent and were PAS-D negative. Preliminary fluorescence microscopy and flow cytometry (n=2) showed increased uptake of BODIPY in patient neutrophils compared to normal controls.

Conclusions

Our data strongly supports correlation of green inclusions in neutrophils to acute liver injury and high mortality rates. The biochemical features suggest neutrophils may either phagocytose necrotic hepatocytes during acute liver injury or accumulate ceroid due to lipid dysregulation. Preliminary studies utilizing BODIPY show promise in detecting and quantifying these inclusions, although further studies are necessary to determine its clinical utility as a prognostic tool.

Poster 21

Nisha Patel, DO

Resident: PGY 2

TCF3: A Sensitive Marker to Differentiate Seminomatous from Non-Seminomatous Germ Cell Tumors.

Mary M Barrett[1], Asif Shahab[1], **Nisha Patel**[1], Qi Yang[1], Loralee McMahon[1], Guang-Qian Xiao[1], Faqian Li[2]

Departments of Pathology, [1]University of Rochester, Rochester, NY, USA; and [2] University of Minnesota, Minneapolis, MN, USA

Background:
For management purposes, testicular germ cell tumors (GCT) can be classified into seminomatous and non-seminomatous types. Following surgical resection, seminomas are generally treated with low-dose radiation, whereas non-seminomatous GCTs are treated with chemotherapy if needed. The difference in treatment regimens makes a correct diagnosis imperative. Although distinction between these two can often be achieved by microscopic morphology alone, ancillary tests may be needed in challenging cases. Currently, no sensitive biomarkers are available for this purpose. T-cell factor 3 (TCF3), a component of the Wnt signaling pathway in embryonic stem cells, plays an important role in control of pluripotent self-renewal and lineage specification. Here we examine the immunohistochemical expression and diagnostic utility of TCF3 in GCTs.

Design:
Fifty cases of testicular GCTs were collected: 23 seminomas, 6 embryonal carcinomas, 1 teratoma, 1 choriocarcinoma, and 19 mixed GCTs. The components of the mixed GCTs were seminoma (n=3), embryonal carcinoma (n=18), yolk sac tumor (n=9), teratoma (n=15), and choriocarcinoma (n=4). On immunohistochemistry, only nuclear staining was considered positive. Staining was graded as negative (<5% of tumor cells stained), 1+ (5-25% positive), 2+ (26-50%), and 3+ (>50%).

Results:
All non-seminomatous components (n=54) exhibited positive nuclear expression of TCF3 (54/54; 100%). The strength of positivity ranged from 1+ to 3+, with the majority of cases showing 3+ staining (33/54; 61%). In contrast, no TCF3 expression was detected in the majority of seminomatous tumor components (24/26; 92%). Two seminomas (2/26; 8%) exhibited weak (1+) and focal positivity (5% and 5-10% of cells, respectively,) for TCF3.

Conclusion:
TCF3 is a highly sensitive marker for separating seminomatous from non-seminomatous GCTs, and may aid in the distinction of these two therapeutically different entities, benefiting clinical patient management.

Poster 22

Bradley N. Mills, MS

Program Year: 2011

Advisor: Marc W. Halterman, MD

Tumor-suppressive potential of dual specificity phosphatase 1 in glioblastoma tumors.

Bradley N. Mills [1,2], Sophia I. Eliseeva [1], Jeanne N. Hansen [1], and Marc W. Halterman [1,3]

Center for Neural Development and Disease [1], Departments of Pathology and Laboratory Medicine [2] and Neurology [3]

BACKGROUND: The dual specificity phosphatase 1 (*DUSP1, MKP-1*) is a stress-induced enzyme that provides feedback inhibition on MAP kinases. While associated with chemoresistance in other cancers, the role of *DUSP1* in glioblastoma remains unsettled. METHODS: To investigate *DUSP1* expression in glioblastoma, we analyzed archived microarray data from the Repository for Molecular Brain Neoplasia Data (REMBRANDT) as well as a cohort of GB tumors from the Rochester Brain Bank. *DUSP1* regulation was studied *in vitro* to define the response to hypoxia, chemotherapy, and differentiation using qPCR. The effects of manipulating *DUSP1* expression on proliferation, apoptosis, and stemness were examined with flow cytometry. RESULTS: *In silico*, *in vivo*, and *in vitro* analyses of GB samples/cells revealed marked variation in *DUSP1* mRNA with levels below control specimens in up to 25% of cases. *DUSP1* mRNA induction was observed *in vitro* upon introduction of disease-relevant factors including hypoxia and chemotherapy. Interestingly, *DUSP1* expression was also found to increase upon cellular differentiation, and enforced *DUSP1* expression induced maturation of GB tumor stem cells. *DUSP1* overexpression was also found to decrease GB tumor stem cell and cell line viability and proliferation. CONCLUSIONS: While part of the observed variability in *DUSP1* expression *in vivo* appears related to stimulatory effects of tumor ischemia and/or chemotherapeutic exposure, acquired mutations of upstream regulators of *DUSP1* and/or *DUSP1* loss of function mutations may be involved. Our findings argue that strategies geared towards increasing *DUSP1* activity *in situ* could augment existing chemotherapeutic approaches.

Poster 23

Allison Li

Program Year: 2014, MSTP

Advisor: Laura Calvi, MD

Restoration of the bone marrow microenvironment improves hematopoietic function in a murine model of myelodysplastic syndrome

Allison J. Li[1], Sophia R. Balderman[3], Benjamin J. Frisch[3], Mark W. LaMere[3], Michael W. Becker[3], Laura M. Calvi[2]

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Myelodysplastic syndrome (MDS) is a hematologic malignancy characterized by bone marrow failure due to ineffective hematopoiesis, leading to significant morbidity and mortality from complications of multi-lineage blood cytopenias and a high risk of transformation to acute leukemia. While many studies have focused on hematopoietic cell-intrinsic aberrations as the etiology of hematopoietic dysfunction, recent reports indicate that cell-extrinsic alterations of the bone marrow microenvironment (BMME) may also contribute MDS pathogenesis. However, the lack of examination of the BMME in a robust *in vivo* model has limited progress in the understanding of reciprocal MDS-BMME interactions. If microenvironmental defects contribute to disease progression, the BM niche may be a beneficial therapeutic target. We studied the MDS-BMME in a well-established transgenic murine model which recapitulates hallmark features of human MDS due to *Vav*-driven hematopoietic-specific expression of the NUP98-HOXD13 (NHD13) fusion gene. BM stroma in NHD13 mice showed increased CD51+/Sca1- osteoblastic cells (OBC) and CD51+/Sca1+ multipotent stromal cells (MSC) compared to WT. While an expansion of MSCs and OBCs could result in skeletal changes, MicroCT imaging of NHD13 hindlimbs revealed no differences in skeletal parameters compared to WT, suggesting that expanded OBCs in NHD13 mice are not functional bone forming cells. Therefore, alterations of these stromal populations which normally play an important role in regulating hematopoietic stem cells (HSCs) may contribute to a dysfunctional BMME. To determine if the MDS-BMME contributes to hematopoietic failure, NHD13-BM was transplanted with WT competitor BM into NHD13 or WT recipients, thus exposing the same MDS-BM to either MDS or WT microenvironments. WT competitor-derived cells exhibited myeloid skewing, a feature of the NHD13 model, when transplanted into NHD13 compared to WT recipients, suggesting that interaction of WT-BM with an MDS-BMME can induce an MDS phenotype. NHD13-BM was next transplanted non-competitively into NHD13 and WT recipients. WT recipients demonstrated improvements in leukopenia and anemia along with increased HSCs compared to NHD13 recipients, suggesting that WT-BMME components can rescue hematopoietic function in MDS. Our studies suggest that a murine model of MDS exhibits marrow microenvironmental dysfunction and that normalization of the microenvironment can improve hematopoietic function in the setting of MDS.

Poster 24

Zachary C. Murphy, MS

Program Year: 2013

Advisor: James Palis, MD

Canonical and non-canonical STAT signaling regulates the maturation of embryonic erythroid cells

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Erythropoietin (EPO), acting through its cognate receptor (EPOR) and downstream JAK2/STAT5 signaling, is the critical regulator of adult (definitive) red cell production. In contrast, both STAT3 and STAT5 are expressed in the transient wave of primitive erythroblasts necessary for survival of the early mammalian embryo. Primitive erythroblasts mature as a semi-synchronous wave in the embryonic circulation, thus providing a useful model system to study JAK2-STAT5/3 signaling. Though, EPO induced activation and nuclear retention of both STAT5 and STAT3 in primitive erythroblasts, STAT5 had varying levels of intensity dependent on the maturational stage of the erythroblast, while STAT3 signaling was maintained during maturation. STAT3 knockdown in primary primitive erythroblasts blocked erythroid maturation, while STAT5 and JAK2 inhibition each appeared to accelerate erythroid maturation. Changes following STAT3 knockdown occur late in both in vitro and in vivo assays suggesting that the role of STAT3 is critical once STAT5 signal intensity has diminished. Since knockdown of JAK2, STAT5, and STAT3 each caused increased apoptosis of maturing primitive erythroblasts, we screened for canonical STAT5 and STAT3 gene targets associated with apoptosis in primitive erythroblasts using ChIP assays. Interestingly, common and uniquely regulated STAT5 and STAT3 genes were identified. Additionally, non-canonical signaling of STAT3 in the context of mitochondria has also been reported. Here we confirm that mitochondria are progressively cleared during erythroid maturation and that the mitochondrial membrane potential also decreases during maturation. Chemical inhibition of STAT3 resulted in rapid loss of mitochondrial membrane potential, suggesting that loss of STAT3 may compromise mitochondrial integrity. However, genetic knockouts of STAT3 using EPOR directed cre-recombinase did not result in increased ROS or decreased mitochondrial polarization. Preliminary evidence suggests that these cells have a stable, truncated N-terminus STAT3 protein, which is reported to be the terminus active non-canonically in mitochondria. Taken together, our data indicate that STAT5 and STAT3 each signal canonically downstream of EPOR to regulate survival and terminal maturation of primary primitive erythroblasts with STAT3 dependent transcription increasing relative to STAT5 through maturation. Additionally, STAT3 may play a non-canonical role in the regulation of mitochondria in primitive erythropoiesis; however, this cannot be addressed using existing STAT3 knockout models.

Poster 25

Cynthia Tang

Program Year: 2015

Ad Hoc Advisor: Robert Mooney, PhD

Developmental and regenerative potentials of skeletal stem cells regulated by Wnt signaling

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The sutures, equivalent to growth plates in the long bone, serve as growth centers critical for healthy development of the craniofacial skeleton. Defects in suture morphogenesis cause premature suture closure, resulting in craniosynostosis, a devastating disease affecting 1 in ~2,500 infants. Recently, we successfully identified and isolated suture stem cells (SuSCs), which are responsible for not only the formation, but also maintenance of craniofacial bones. Upon injury, SuSCs react rapidly and contribute directly to bone repair by replacing the damaged tissue, and in a calvarial injury model, bone healing is highly facilitated by SuSC transplantation. Although the suture mesenchyme has been postulated to act as a niche for SuSCs, little is known about SuSC regulation, which greatly restricts our further understanding of mechanisms driving craniofacial disease pathogenesis. The expression of Axin2 by SuSCs leads us to hypothesize they are regulated by Wnt signaling. Here we investigate if Wnt provides an essential signal for maintaining SuSC stemness. Microarray and RT-PCR analyses identify Wnt ligands showing differential expression in SuSCs. We examine the expression pattern of these candidates and their relative localizations in the niche, and new findings support our initial hypothesis for Wnt signaling activation in SuSC maintenance. To definitively assess the functional requirement of Wnt, we inactivate *Gpr177*, the mammalian orthologue of *Drosophila Wls/Evi/Srt*, to omit Wnt secretion from signal-producing cells *in vivo*. Mice exhibit severe skull deformities. Future studies aim to examine how SuSC-mediated skeletal repair and regeneration are affected by the mutation. Future studies will also investigate autocrine and paracrine as well as canonical and non-canonical effects of Wnt in the self-renewal, clonal expansion, proliferation, and differentiation abilities of SuSCs. These experiments will provide thorough examinations into the regulation of SuSCs by Wnt and the necessity of Wnt for the maintenance of suture structure and prevention of craniosynostosis.

Poster 26

Katherine Best

Program Year: 2015

Ad Hoc Advisor: Robert Mooney, PhD

Assessing the Role of Scleraxis Lineage Cells during Murine Flexor Tendon Healing

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Flexor Tendon (FT) injuries affect more than one million people each year. Satisfactory healing is impeded by exuberant deposition of extracellular matrix, resulting in the formation of range of motion-limiting scar tissue. Despite the prevalence of these injuries, little is known about the molecular mechanisms involved in FT healing and currently no satisfactory treatment options are available. mRNA expression of Scleraxis (*Scx*), which is required for normal tendon formation, is increased following FT injury. We hypothesized that *Scx* lineage cells are localized to the granulation tissue following injury. To test our hypothesis, Scx-Cre; nT/nG mice were utilized as a tendon reporter, with Scx lineage cells expressing GFP. These mice received a unilateral FT transection between 10-12 weeks of age. At 10 and 14 days post-surgery, injured and sham hindpaws were harvested and imaged using fluorescent microscopy. Post-surgery FTs revealed Scx lineage cells are restricted to the native tendon and are absent from the granulation tissue, suggesting that *Scx* lineage cells do not directly participate in scar tissue formation at the site of injury. However, the potential indirect role of Scx in this process is not yet known. In healing FTs, the density of *Scx* lineage cells appears highest at the epitenon-tendon boundary, suggesting that peripheral cells may have a greater role in FT repair than cells within the body of the FT. Additionally, preliminary immunohistochemistry experiments detected Scx protein localization along the periphery of the tendon near the site of injury. Taken together, we provide evidence that Scx lineage cells are localizing, either through proliferation or migration, at the periphery of the tendon following FT injury, explaining the localization of Scx at or near the epitenon. This discovery is an important first step in elucidating the cellular composition of FT repair.

Poster 27

Robert D. Maynard

Program Year: 2014

Advisor: Cheryl Ackert-Bicknell, PhD

Functional Validation of a Key Bone Mineral Density Locus Determined by Genome-wide Association Studies

Robert D. Maynard[1], Fernando Rivadeneira[2], Carolina Medina-Gomez[2], Kwangbom Choi[3], Cheryl L. Ackert-Bicknell[1]

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Genome-wide association studies (GWAS) for bone phenotypes have aided in the discovery of novel loci, but functional validation is lacking for many of these novel loci. GWAS have repeatedly identified a single nucleotide polymorphism (SNP) in the locus 7q31.31 that is significantly associated with bone mineral density (BMD) and aligns with the uncharacterized gene *CPED1.* Our data has shown that this gene is widely expressed in mouse tissues, and relevant to bone, we have determined that *Cped1* is expressed in mouse cortical bone, calvarial osteoblasts, and in the multipotent C3H10T1/2 cells when they are differentiated with osteogenic media. Conversely, no expression was observed in the macrophage line, RAW264.7, which can be stimulated down the osteoclast lineage. In maturing calvarial osteoblasts, the pattern of expression of *Cped1* is highly correlated with other extracellular matrix proteins such as the glycoprotein Osteonectin (*Sparc,* R^2 = 0.99*)* and Type I collagen (*Col1a1*, R^2 = 0.83). We have established that the 3rd exon is alternately spliced out resulting in at least 2 transcripts and thus 2 putative protein products. The expression of both isoforms increases during osteoblastogenesis. To understand the role of these isoforms in osteoblast function, siRNAs targeting either exon 1 (present in both transcripts) or exon 3 (isoform specific) were delivered to C3H10T1/2 cells concurrent with osteogenic media, and cells were differentiated for up to 10 days. By day 5, cells treated with siRNA against exon 1 had reduced alkaline phosphatase (ALP) staining, and this coincided with a 99% reduction in message. Interestingly, *Cped1* exon 3 siRNA led to increased ALP staining and an 85% increase in expression, suggesting that these *Cped1* isoforms are not redundant in function. A similar observation was made on day 10 post knockdown. In summary, these data strongly suggest that the two known isoforms of this gene play opposing roles in osteoblast maturation and that this gene may have a key role in bone biology.

Poster 28

Madison Doolittle

Program Year: 2015

Ad Hoc Advisor: Robert Mooney, PhD

Investigating the Genetic Regulation of Osteoporosis through Validation of Genes Associated with Variation in Bone Mineral Density

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Bone mineral density (BMD) is a clinical measurement of mineralized tissue used for the diagnosis of bone pathologies such as osteoporosis, osteopenia, and osteogensis imperfecta. Low BMD is an indicator for osteoporosis and fracture risk and may predict susceptibility to major fractures, which have been shown to cause significantly higher morbidity and mortality. BMD is influenced by a multitude of factors. However, overwhelming evidence advocates that BMD is a highly heritable trait where up to 85% of the variance in peak bone mass is accredited to genetics alone. Multiple genes, most of which are yet to be discovered, contribute to BMD variation through creating imbalances between bone formation and resorption. To investigate this polygenic disorder, Genome Wide Association Studies (GWAS) are implemented to identify loci at which there exists a genetic variation correlating with a specific phenotype. In GWAS testing for BMD, a large number of single nucleotide polymorphisms (SNPs) have been identified at the locus 1q36.12, which resides within the Zinc Finger and BTB Domain-containing 40 gene (ZBTB40). These zinc finger genes code for small protein structural motifs that are involved in interactions with DNA, RNA, and proteins, allowing for various therapeutic and research manipulations. ZBTB40 has no defined function, however this statistical association suggests involvement in BMD regulation. To determine the biological function, we ran RNA and protein profile analysis in C3H10T½ osteoblast progenitor cells, revealing that ZBTB40 has high expression early in development, then decreases and plateaus as the cells differentiate and mature. Subsequent siRNA knockdown of exon 17 of ZBTB40 in C3H10T½ cells produced intensification of Alkaline Phosphatase expression, suggesting an increase in osteoblast maturation and proliferation. Because of the decrease in expression as the cells mature, and increase in osteoblast proliferation upon knockdown, we believe that ZBTB40 may have an inhibitory and regulatory effect on osteoblast function and thus have an influence on BMD regulation and its related bone pathologies.

Poster 29

Sarah E. Catheline, MS

Program Year: 2013

Advisor: Jennifer Jonason, PhD

IKKβ Activation in Postnatal Chondrocytes Results in a Chronic Inflammatory Environment within the Knee Joint and Recapitulates an Age-Associated Osteoarthritis Phenotype

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Osteoarthritis (OA) is a degenerative joint disease that leads to loss of cartilage within the synovial joints. Aging is an important contributing factor to the development of OA, and age-related increases in low-grade inflammation are thought to be detrimental to cartilage health. Here, we present data demonstrating that canonical NF-κB signaling is active in the cartilage of aged (27-month-old) wild type C57BL/6J mice with osteoarthritic knee joints. Since NF-κB has been implicated in regulation of collagenase and aggrecanase gene expression, key molecules responsible for the breakdown of cartilage ECM, we hypothesize that activation of canonical NF-κB signaling in postnatal chondrocytes will be sufficient to drive the onset of OA. To test this hypothesis, we generated mice that overexpress a constitutively active form of IKKβ in specifically postnatal chondrocytes following administration of tamoxifen at 2 months of age. At 8 months of age, histological analysis reveals that the knee joints of these genetically altered mice remarkably phenocopy those of the aged wild type mice. Specifically, we observe proteoglycan staining loss in the articular cartilage, synovial hyperplasia, and an enlarged meniscus within the knee joints of both groups. Further characterization of the IKK mutant mice reveals decreased staining for PRG4 within the articular cartilage, meniscus and synovium. MMP13-positive cells and increased Alizarin red staining are also found within the enlarged meniscus of these mice, suggesting that the native ECM of the meniscus is undergoing degradation and mineralization. Finally, the IKK mutant mice show an increase in COX-2 positive cells within the articular cartilage and meniscus, further supporting a local inflammatory environment existing within the joints of these mice. Based on the observed phenotypes, this IKKβ activation model represents a novel murine model of aging-induced OA.

Poster 30

Richard D. Bell

Program Year: 2014

Advisor: Edward M. Schwarz, PhD

Early Onset Morbidity and Mortality in Female TNF-Tg Mice with Inflammatory-Erosive Arthritis and Interstitial Pulmonary Disease

Richard D. Bell[1,2], Emily K. Wu[2,3], Ronald W. Wood[2,4,5], Joe V. Chakkalakal[1,2], Javier Rangel-Moreno[1], Maria de la Luz Garcia-Hernandez[1], Christopher T. Ritchlin[6], Edward M. Schwarz[1,2,3,5] and Homaira Rahimi[2,7]

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Women have a greater prevalence of Rheumatoid arthritis (RA) compared to men, and women with RA have an increased risk of cardiovascular and respiratory mortality compared to women without RA. However, the etiology of sex differences and cause of cardiopulmonary disease in RA remains unclear. Tumor necrosis factor-transgenic (TNF-Tg) mice, a model of inflammatory-erosive arthritis, also display similar sex differences in arthritis severity and mortality but the cause of the sexual dimorphism is unknown. Thus, the goal of this study was to investigate the sexual dimorphic etiology of both arthritis and mortality in TNF-Tg animals. Six, 5.5-month female TNF-Tg animals were sacrificed for necropsy analysis. The histopathology report and immunostaining indicated sever infiltration of B-Cells, T-cells, and macrophages into the interstitium of the lung, pulmonary arteriosclerosis and enlarged right ventricles. To quantify the infiltrate within the lung, 12-month old male TNF-Tg and wildtype (WT, n=6) lungs were collected, stained with CD3, CD19, CD11b and CD11c, and cell numbers were assessed by flow cytometry. CD3+(3.2±1.9vs1.2±1.0), CD19+(3.7±1.9vs0.4±0.4) and CD11b/CD11c+(4.2±4.2vs0.3±0.2) cells showed increased numbers in TNF-Tg animals compared to WT littermates ($p<0.05$, cells $x10^4$). Furthermore, young female and male TNF-Tg and WT animals were tested for grip-strength, a measure of arthritis progression, and weight at 2- and 3-months old. Female TNF-Tg animals displayed decreased grip-strength at 2- and 3-months compared to male TNF-Tg animals and the WT littermates (2-months:1.7±0.4, 2.4±0.2, 2.2±0.2, and 2.5±0.3; 3-months:1.3±0.3, 2.0±0.2, 2.6±0.1, and 2.5±0.2; $p<0.01$). Female TNF-Tg animals also fail to gain weight from 2 to 3 months compared male TNF-Tg and WT littermates (2±2%, 7±3%, 10±6%, and 8±4%, $p<0.05$). These data indicate that female TNF-Tg mice have increased morbidity and sever inflammatory interstitial lung disease that likely contributes to increased mortality. This is the first study to identify the eitology of sex differences in the TNF-Tg animal model.

Poster 31

Eric Schott, MS

Program Year: 2013

Advisor: Robert Mooney, PhD

Manipulation of the Gut Microbiome: A Potential Therapeutic Effect on Osteoarthritis in Obese Mice.

Eric Schott[1,2]**,** Christopher W Farnsworth[1,2], Madison Doolittle[1,2], Jun Zhang, Raul Gonzalez[1], Steven Gill[3], Robert Mooney[1,2], Michael Zuscik[1,2].

Affiliation: 1. Department of Pathology and Laboratory Medicine, UR Medical Center, Rochester NY. 2. Center for Musculoskeletal Research, UR Medical Center, Rochester NY. 3. Department of Microbiology, UR Medical Center, Rochester NY.

Obesity-induced type 2 diabetes (T2D) is a major risk factor for the development of osteoarthritis (OA), as 47.3% of T2D patients have arthritis, and 66% of individuals with OA are either obese or obese/T2D. Despite the known association, the mechanism linking this comorbid condition has not been fully elucidated. Our recent data suggest that in obesity/T2D increased systemic inflammation may play a major role in disease progression. Both published and preliminary data establish that synovium from obese/T2D patients is inflamed with an increased presence of macrophages and monocytes. Intriguingly, recent evidence suggests that in obesity/T2D, the gut microbiome is altered, with an increase in bacterial phyla associated with inflammation and lymphocyte activation, leading to other pathologic states. Studies have also shown that this obese gut microbiome can be altered by prebiotic supplementation, reverting the bacteria to those found in a lean, healthy gut. These findings lead us to hypothesize that prebiotic supplementation may provide protection from OA in obese/T2D mice. To address this hypothesis, mice were fed a lean or high fat diet for 12 weeks, then switched to a lean or high fat diet supplemented with a control non-digestible fiber (cellulose) or experimental prebiotic (oligofructose). After 2 weeks on the new diet, OA was surgically induced by a meniscal injury, and the mice were continued on the supplemented diets for 12 weeks. Obese mice supplemented with oligofructose had improved glucose tolerance, decreased serum cytokine levels, decreased serum endotoxin levels, and improved bone mineral density (BMD), all while maintaining an equal weight, and similar body fat percentage to those on the control cellulose supplement. These measurements agree with our hypothesis, and provide rational to continue tissue analysis from these animals. Joints will be analyzed for OA by microCT, OARSI scoring, cartilage histomorphometry, and immunofluorescent staining for cytokine and catabolic enzyme content. From this study, we will determine whether manipulation of the gut microbiome can protect obese/T2D and or lean mice from OA.

Poster 32

Xi Lin

Program Year 2015

Advisor: Lianping Xing, PhD

Proteasome inhibition is a potential treatment for osteoarthritis by attenuating inflammation and improving lymphatic function

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Osteoarthritis (OA) is a whole joint disease associated with chronic inflammation in synovium and surrounding soft tissues. Various cell types participate in OA pathogenesis by producing catabolic factors that are accumulated in the joint space to accelerate tissue damage. We have reported that the synovial lymphatic system (SLS) removes catabolic factors and inflammatory cells from arthritic joints, associated with reduced lymphatic draining function in OA. However, how inflammation affects the SLS, the cellular and molecular mechanisms involved, and if its inhibition could be a benefit in OA have not been studied. We hypothesize that OA is associated with inflammatory M1 macrophage (M1)-mediated SLS dysfunction due to elevated protein ubiquitination and degradation, which can be attenuated by Bortezomib (Btz), a clinically used proteasome inhibitor. To test this hypothesis, we used Meniscal-ligamentous injury-induced mouse model of OA in WT C57BL/6 mice. First, we performed RNAseq and pathway analysis in purified lymphatic endothelial cells (LECs) isolated from OA synovium and found highly activated inflammatory pathways. Flow analysis indicated significantly increased percentage of M1s, but not M2s, in OA vs. sham synovium (F4/80+CD86+ M1s: 1.89±0.35 vs. 0.80±0.19%; F4/80+CD36+ M2s: 1.35±0.29 vs. 1.52±0.15%). IHC confirmed increased M1s, but not M2s, in OA and some of M1s are localized adjacent to lymphatic vessels. Secondly, we co-cultured M1s or M2s with LECs and demonstrated that M1s induced expression of TNF (8-fold), IL-1 (10-fold), iNOS (40-fold) in LECs. Next, we examined ubiquitinated (Ub) proteins by Western blot and found that OA synovium and M1s had markedly increased total Ub proteins (1.5-fold, and 3.2-fold, respectively). Finally, we treated M1s and OA mice with Btz and found that Btz inhibited M1-induced TNF, IL-1 and iNOS expression in LECs. More importantly, intra-articular injection of Btz significantly reduced changes in synovial volume by ultrasound (3.61±0.41 vs. 4.64±0.65 mm^3 with DMSO) and OA tissue damage by histology (OARSI score: 1.7±1 vs. 3.5±2 with DMSO), and increased synovial lymphatic clearance by near-infrared lymphatic imaging (65.2±6.1 vs. 48.2±9.3% with DMSO). In conclusion, OA-associated M1s affect SLS, which can be attenuated by Btz. Proteasome inhibition may represent a new therapy for OA by controlling inflammation and improving SLS function.

Poster 33

Christopher W. Farnsworth, MS

Program Year: 2012

Advisors: Robert Mooney, PhD and Michael Zuscik, PhD

S. aureus adaptation in obesity and T2D in orthopaedic infection is mediated by ClfA

Christopher W. Farnsworth[1,2], Eric Schott[1,2], Sarah Jensen[1], Cindy Shehatou[1], Michael Zuscik[2], Robert A. Mooney[1,2].

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Background: Obesity and type 2 diabetes (T2D) are known risk factors for *Staphylococcus aureus* infection following orthopaedic surgery. Despite this, little is known about the mechanism(s) of increased infection rates in obese/T2D patients. Recent work has demonstrated that *S. aureus* utilizes host clotting proteins, including fibrinogen, to evade host immune responses. Furthermore, T2D patients are well known to have clotting defects and hypercoagulation. Here, we test the hypothesis that *S. aureus* adapts to the altered host environment in T2D to increase virulence. **Methods:** Obesity and T2D were induced in mice using a diet composed of 60% Kcal from fat, or a control diet of 10% Kcal from fat. Mice were then given a trans-tibial implant infected with WT *S. aureus* or a mutant strain, ΔClfA. ClfA is a *S. aureus* fibrinogen binding protein which is crucial in early bacterial protection and abscess formation. 14 or 21d post infection, bacterial loads were quantitated by CFU's or histological staining was performed. In vitro *S. aureus* growth and fibrin deposition was measured in mouse and human plasma using 96 well plates and measuring optical density over time. **Results:** RNAseq of *S. aureus* isolated from the infected mice indicated a 34-fold increase in ClfA expression in *S. aureus* isolated from T2D mice. Furthermore, T2D mice have increased infection severity compared to lean mice by CFU's and quantitation of *S. aureus* abscess communities by histology. These results were attenuated in T2D mice infected with ΔClfA *S. aureus* but not lean mice. In vitro, *S. aureus* metabolic activity and fibrin deposition were elevated in plasma from T2D mice and patients compared to non-diabetic controls. **Conclusion:** *S. aureus* upregulates ClfA gene expression in T2D hosts. This correlates with an increase in bacterial burden and *S. aureus* abscess formation in T2D mice. Bacterial burden was attenuated in T2D mice only by infecting with a mutant *S. aureus* strain deficient in ClfA. This demonstrates a potential therapeutic regimen specific to T2D patients using anti-ClfA antibodies as a passive immunization prior to orthopaedic surgery with the goal of reducing infection rates.

Poster 34

Melissa Glasner

Program Year: 2015

Advisor: Alayna Loiselle, PhD

A Murine Model of Type 2 Diabetes Compromises Biomechanics and Gliding Function of Flexor Tendons

Melissa Glasner[1,2], Jessica Ackerman[2], Fatima Bawany[2], Alayna Loiselle[2]

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Approximately 50% of Type II Diabetics will be diagnosed with Diabetic Hand Syndrome (DHS). DHS is characterized by restricted digit range of motion due to impaired gliding function of the flexor tendons. Impaired tendon gliding function is in part due to alterations in collagen fiber organization, which is normally composed of type I collagen. Clinically, diabetic tendons are stiffer, weaker, more likely to rupture, and have an impaired healing response. Despite this clinical burden, there are no treatment options to improve tendon function in these patients. In the present study, we tested the hypothesis that a murine model of diet-induced obesity and Type II Diabetes (T2DM) would recapitulate the progressive diabetic tendinopathy phenotype observed clinically. We observed that diabetic flexor tendons have impaired gliding function, increased stiffness, and a decrease in max load at failure over time, relative to lean controls. Histologically, collagen in the diabetic tendon is disorganized and exhibits increased deposition of Type III collagen, indicating blunted extracellular matrix remodeling. Additionally, we tested if reversal of T2DM would rescue these progressive pathological changes in tendon. However, reversal of T2DM was not sufficient to rescue the biomechanical and collagen disorganization phenotypes we observed in diabetic tendon. Taken together, the present study demonstrates that a murine model of diabetic tendinopathy phenocopies many aspects of the clinical presentation of DHS. This model displays, increased tendon stiffness, loss of collagen matrix organization, and impaired gliding function. Additionally, it can be concluded, that restoration of normal metabolism is insufficient to reverse these phenotypes. Thus, this novel animal model will facilitate our understanding of the cumulative cellular and molecular changes that occur in diabetic flexor tendons, and will aid in the identification of therapeutic targets

Poster 35

Chi-Wen Lo, MS

Program Year: 2012

Advisor: Jan Czyzyk, MD

The role of serpin B13 antibodies in type 1 diabetes mellitus

Chi-Wen Lo, Yury Kryvalap, Raman Baldzizhar, Nadzeya Kryvalap, Jan Czyzyk

Department of Pathology & Laboratory Medicine

Successful therapy for type 1 diabetes (T1D) requires both suppression of autoimmune inflammation and restoration of beta cell mass. Our recently published work demonstrated that exposure to endogenous autoantibodies or the exogenous monoclonal antibody (mAb) against serpin B13 protease inhibitor is associated with diminished anti-insulin antibody production, reduced T-cell infiltration of pancreatic islets and delayed onset of diabetes in T1D-prone NOD mice. Additional studies in Balb/c mice revealed that exposure to these antibodies is associated with increased islet number and mass, and improved metabolic control after induction of diabetes with streptozotocin. Therefore, anti-serpin B13 antibodies not only reduce autoimmune inflammation but also promote regenerative changes in pancreatic islets. To investigate the mechanism of islet regeneration by anti-serpin B13 antibodies, we used lineage-tracing and nucleotide analog incorporation approaches to examine beta cell neogenesis and proliferation, respectively. We found that there are more proliferation events in the small mice receiving anti-serpin B13 antibodies. At the same time we did not observe generation of new beta cells from either Hnf1b+ ductal cells or Ngn3+ progenitor cells. In addition, Reg genes, which are associated with islet proliferation and development, were up-regulated in anti-serpin B13 Ab treated mice. We also found that adding serpin B13 mAb to in vitro cultures containing both pancreatic islets and extract of the pancreatic ductal epithelium (in which serpin B13 is expressed) leads to significant up-regulation of Reg gene expression and islet cell proliferation. Finally, the protease activity of cathepsin L, which is the known target of serpin B13, was enhanced in the pancreatic lysate containing anti-serpin B13 mAb. These results suggest that islet regeneration induced by anti-serpin B13 antibodies is due to beta cell proliferation rather than neogenesis. In addition, our preliminary studies implicate Reg genes and proteases (e.g.catepsin L) involved in the beta cells proliferation enhanced by serpin B13 mAb.

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Poster 36

Shana Straub, MD

Resident: PGY 3

PLZF: A Sensitive and Specific Biomarker for Yolk Sac Tumor

Shana Straub[1], David Burstein[2], **Hani Katerji[1]**, Faqian Li[3], Qi Yang[1], McMahon Loralee[1], Guang-Qian Xiao[1]

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Background: Germ cell tumors (GCTs) differ in their clinical behavior and management. Yolk Sac Tumor (YST) has been known to be the most commonly underdiagnosed GCT element. The currently available GCT biomarkers are only marginally sensitive and/or specific for YST. Although the function of Promyelocytic Leukemia Zinc Finger (PLZF) protein, a transcription repressor, is well studied in spermatogenesis, expression of PLZF in GCT has not yet been reported. The aim of this study was to investigate the immunohistochemical expression and diagnostic utility of PLZF in GCTs.

Design: A total of 67 adult GCTs collected at University of Rochester Medical Center were studied, which included 62 testicular primary GCTs, 2 ovarian YSTs, 1 mediastinal YST, and 2 retroperitoneal metastatic testicular YSTs. Of the 62 testicular primary GCTs, 34 were pure GCTs (20 seminomas, 8 embryonal carcinomas (ECs), 2 teratomas, 1 choriocarcinoma, 1 monodermal carcinoid, and 2 spermatocytic seminomas) and 28 were mixed GCTs (composed of 13 ECs, 15 YSTs, 15 teratomas, 7 seminomas, and 3 choriocarcinomas in a various mixture). 35 cases contained intratubular germ cell neoplasia (ITGCN). Immunoreactivity was graded as: <1% (negative), 1-25% (focal), 25%-50% (moderate), and >50% (diffuse) staining.

Results: YST was consistently reactive with PLZF. Among the 15 testicular YSTs in mixed GCTs, all (100%) presented with moderate to diffuse PLZF staining. PLZF reactivity was present in all the growth patterns of YST. PLZF also picked up small foci of YST intermixed/embedded in other GCT subtype elements of mixed GCT. Additionally, diffuse PLZF immunereactivity was observed in 2/2 recurrent metastatic YSTs, 1/1 mediastinal YST, and 2/2 ovarian YSTs. Except spermatocytic seminoma (n=2) and carcinoid (n=1), in which PLZF was also diffusely expressed, all the other non-YST GCTs were completely nonreactive with PLZF, including seminoma (n=27), EC (n=21), teratoma (n=17), choriocarcinoma (n=4), and ITGCN (n=35). The sensitivity and specificity of PLZF in detecting YST was 100% (20/20) and 96% (66/69), respectively.

Conclusion: Our study demonstrated that PLZF moderately to diffusely immunoreacted with all YSTs (gonadal, extragonadal and metastatic) and, except in spermatocytic semnioma and carcinoid, no immunoreactivity was observed in the other types of GCTs. In conclusion, PLZF is a highly sensitive and specific marker for YST, superior to other currently available YST biomarkers - alpha-fetoprotein and Glypican 3.

Poster 37

Shana Straub, MD

Resident: PGY 3

Comparison of Dysplastic Fundic Gland Polyps in Patients With and Without Familial Adenomatous Polyposis

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Background: Fundic gland polyps (FGPs) are the most common gastric polyp. Dysplastic FGPs (d-FGPs) are rare; they typically arise in patients with familial adenomatous polyposis (FAP) but may occur in non-syndromic (NS) patients. They almost never progress to carcinoma, but their significance is unclear, especially in NS patients. The APC/β-catenin pathway has been implicated in their pathogenesis, and β-catenin immunohistochemistry (IHC) is occasionally positive. We compared the clinicopathologic and IHC findings of d-FGPs in FAP and NS patients.

Materials and Methods: We identified 115 FGPs diagnosed with low-grade dysplasia (LGD) or high-grade dysplasia (HGD) or deemed indefinite for dysplasia (IFD) from 63 patients (28 with clinical FAP, 35 NS). We recorded patient sex, age at first d-FGP, time until subsequent d-FGP (if any), history of non-gastric cancer (no patients had gastric cancer), proton-pump inhibitor (PPI) use, and presence of *Helicobacter pylori*. β-catenin IHC was performed on cases with available blocks, with any nuclear staining considered positive.

Results: Median age at d-FGP diagnosis was 31 years for FAP patients and 59 years for NS patients (P<0.0001). Patients were commonly female (15/28 FAP [54%], 25/35 NS [71%], P=0.1903). Sixteen FAP patients (57%) developed at least one subsequent d-FGP, compared to 8 (23%) NS patients (P=0.0087). Median time between d-FGP detection was 12 months in FAP patients and 7 months in NS patients (P=0.8221). PPI use (usually omeprazole) was seen in 13 FAP patients (46%) and 28 NS patients (80%) (P=0.0079). Only one patient (who was NS) had *Helicobacter*. Seven FAP patients (25%) and 13 NS patients (37%) had reported non-gastric malignancies (P=0.4155). The 115 FGPs included 97 with LGD, 3 with HGD, and 15 IFD. β-catenin IHC was positive in 12/103 (12%), including 10/86 with LGD, 2/3 with HGD, and 0/14 IFD. It often highlighted small dysplastic foci and demonstrated non-dysplastic surface epithelium overlying dysplastic nests. Three of 28 FAP patients (11%) and 6/35 NS patients (17%) had an IHC-positive polyp (P=0.7192). Two FAP patients and one NS patient had more than one IHC-positive polyp.

Conclusions: FAP patients unsurprisingly are diagnosed with d-FGPs earlier in life and are more likely to develop additional ones compared to NS patients. FAP and NS patients with multiple d-FGPs experience a roughly similar length of time between their detection. If PPIs play a role in d-FGP development, this effect is more prominent in NS patients. Dysplastic FGPs in FAP and NS patients have similar low rates of β-catenin IHC positivity. FAP and NS patients developed non-gastric cancers at similar rates, suggesting that d-FGPs may portend a general increased risk of carcinogenesis in NS patients.

Poster 38

Robert Hoff, MS

Program Year: 2012

Advisor: Dirk Bohmann, PhD

Characterizing Nrf2 Signal Responsiveness during Aging using Fluorescent Reporters

Robert Hoff[1,2], Dirk Bohmann[2]

[1]Department of Pathology and Laboratory Medicine, [2]Department of Biomedical Genetics

Age is the leading risk factor for chronic diseases that account for the bulk morbidity and mortality. Experimental work in model organisms and humans suggests a defining characteristic of aging is a failure to adapt to various forms of stress, such as oxidative stress and exposure to toxic agents. At the cellular level, the response to stress is mediated by signal responsive transcription factors that modulate gene expression to restore homeostasis. Nrf2 is one such stress responsive transcription factor and functions as a master regulator of the antioxidant response. Our lab has found an age related loss of Nrf2 mediated induction of antioxidant genes in response to stress. We hypothesize that loss of inducibility of Nrf2 target genes happens gradually during aging due to irreversible changes in chromatic organization at individual gene loci in the genome. To test this, we have generated transgenic Drosophila expressing two different fluorophores (nuclear localized GFP and dsRed) under the control of Nrf2 response elements in the genome (Antioxidant Response Element-ARE) using site-specific integration. We predict that both reporters should be equally responsive in cells of young tissue, but that differential expression patterns will be observed in old animals due to stochastic loss of inducibility of one or both reporters in a cell by cell manner. This system will enable us to address fundamental questions in aging research and allow for detection of factors that modulate the aging process.

Poster 39

Min Tian, MS

Program Year: 2010

Advisor: Dirk Bohmann, PhD

Drosophila Cnc proteins regulates both antioxidant response and endoplasmic reticulum stress

Min Tian[1,2], Dirk Bohmann[1]

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Cellular stress response is a critical mechanism which protects organisms from damage and restores cellular homeostasis. Improper stress response often contributes to disease progression and increased stress resistance promotes longevity in numerous animal models. Redox homeostasis in animal cells is controlled in a large part by a family of transcription factors represented by Cnc in *Drosophila* and Nrf family in mammals. Cnc and Nrf2 have well-established roles in promoting redox homeostasis and small molecule detoxification. Our study shows that *Drosophila* CncC is localized in endoplasmic reticulum via the N-terminal motif NHB1. Also, beside the role in regulating oxidative stress defense, our data suggest CncC is also important in ER stress response. Over all, our study suggests that Cnc is *Drosophila* homolog for both Nrf1 and Nrf2 and CncC protein is an important for the stress defense mechanisms for both oxidative stress and ER unfolded protein response.

Poster 40

Benjamin A. Plog, MS, MSTP

Program Year: 2012

Advisor: Maiken Nedergaard, MD

Impaired cerebral arterial pulsatility drives glymphatic failure, neuroinflammation, and neurobehavioral deficits following decompressive craniectomy

Benjamin A. Plog[1,2,3], Nanhong Lou[2], Clifford A. Pierre[2], Emi Hitomi[2], Stephen P. Miranda[2,3], Nami Shah[2,3], Hongyi Kang[2], Jeffrey J. Illiff[2,4], Douglas M. Zeppenfeld[2,4], Maiken Nedergaard[2], G. Edward Vates[2]

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Decompressive craniectomy is often a life saving intervention for TBI or stroke patients with refractory cerebral edema. Protracted durations of craniectomy, however, can result in a condition characterized by headaches, dizziness, mood changes, seizures, and focal neurologic deficits. Interestingly, the signs and symptoms of this syndrome can be rapidly and completely reversed by cranioplasty. While the etiology of this condition is poorly understood, altered CSF hydrodynamics have been proposed as a pathogenic mechanism. Recent work from our lab has described the role of the glymphatic system as a physiologic pathway for CSF-ISF exchange and the clearance of interstitial solutes from the brain parenchyma. The objectives of the current study were three-fold: 1) to determine if craniectomy could induce both acute and chronic glymphatic failure, and if cranioplasty could reverse this suppressed function, 2) to elucidate the mechanism of glymphatic inhibition secondary to craniectomy, and 3) to determine if impaired glymphatic dynamics could in turn trigger the neurobehavioral sequelae of the syndrome of the trephined. Acute glymphatic influx and clearance were evaluated with fluorescence microscopy by injecting AlexaFluor-555 conjugated ovalbumin to the cisterna magna of wildtype male mice under control conditions or after a craniectomy procedure. Similarly, chronic glymphatic influx, as well as GFAP, CD68 and AQP4 expression were investigated 14, 28 and 56 days following craniectomy or cranioplasty. Using 2-photon microscopy, the cortical vasculature was initially imaged through a thin skull, which was subsequently converted to an open craniectomy, and the pulsatility index was quantified. Motor and cognitive performance was determined with the rotarod and novel object recognition tests, respectively, at baseline and 14, 21 and 28 days following craniectomy or cranioplasty. It was found that craniectomy induced acute and chronic glymphatic failure, likely as a consequence of decreased penetrating arterial pulsatility. Additionally, chronic glymphatic inhibition was associated with widespread astrocytic and microglial inflammation and a dysregulated AQP4 expression pattern. Finally, impaired glymphatic function was associated with motor and cognitive decline. We conclude that while decompressive craniectomy is often a life-saving maneuver, shorter durations will likely improve patient outcomes by restoring glymphatic CSF flow.

Poster 41

Savador Peña, MS, MSTP

Program Year: 2011

Advisor: Keith Nehrke, PhD

Hypoxia and the Mitochondrial Unfolded Protein Response

Salvador Peña[1], Teresa Sherman[2], Andrew Wojtovich[2], Sergiy Nadtochiy[3], Cole Haynes[4], Paul Brookes[3], Keith Nehrke[4]

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The mitochondrial unfolded protein response (UPRmt) is a surveillance pathway induced by diverse stressors including reactive oxygen species, respiratory chain deficits, and pathologic bacteria. The transcription factor ATFS-1 is required for UPRmt signaling and its trafficking to the mitochondria and the nucleus regulates its signaling output. Consistent with a central role for mitochondria in mammalian ischemia-reperfusion injury, we found that either pharmacological or genetic activation of the UPRmt was sufficient to protect worms from anoxic death and cell damage. We found that *atfs-1* was necessary for this protection. Additionally, a gain-of-function (gf) mutation in *atfs-1* endogenously protected against anoxia. We then created a genetic model to study the cell specific effects of *atfs-1*. Our model utilizes a FLP-FRT-wCherry promoter interruption of an *atfs-1* single copy MosSCI transgene to restrict the expression of either the wild type or gof allele to specific cells. Expression in this model via the native promoter at physiological levels is expected to more closely recapitulate endogenous regulatory mechanisms. Since mitochondrial stress has been suggested to result in non-cell autonomous signaling, we were motivated to use our model to determine whether the UPRmt could also protect cells from anoxic damage through similar mechanisms. We tested whether selective expression of *atfs-1* was sufficient to activate the UPRmt in specific tissues and whether this elicited global adaptation to anoxia. In addition to allowing us to rigorously define the role of *atfs-1* in regulating anoxic sensitivity, this model will be useful in studying how UPRmt influences other cellular and systems outputs.

Poster 42

Pei-Lun Weng, MS

Program Year: 2011

Advisor: Catherine Ovitt, PhD

Non-neuronal lineage derived from Ascl3-expressing cells in homeostasis and regeneration of the olfactory epithelium

Pei-Lun Weng[1], Mridula Vinjamuri[2] & Catherine E. Ovitt[2]

[1]Department of Pathology and Laboratory Medicine, [2]Center for Oral Biology and Department of Biomedical Genetics, University of Rochester School of Medicine and Dentistry, Rochester, New York

The olfactory epithelium (OE) is composed primarily of olfactory sensory neurons (OSNs) and sustentacular supporting cells. Interspersed throughout the OE are less abundant non-neuronal support cells. The origin and replacement of ORNs is well characterized, but the source of the less abundant support cells in the OE is not. We observed that the transcription factor Ascl3 is expressed in the adult OE. Lineage tracing experiments revealed that Ascl3 expression marks bipotential precursors of microvillar cells and Bowman's gland ducts during development. Following chemically-induced injury, we showed that Ascl3 is activated in a subset of horizontal basal cells (HBCs), which repopulate all microvillar cells and Bowman's gland ducts during OE regeneration. Specific ablation of the Ascl3-expressing cells demonstrated that absence of the non-neuronal support cells disrupts OE homeostasis and regeneration, and is linked to a decrease in globose basal cells (GBCs). Our data provide a novel model for investigation of non-neuronal cell contribution to maintaining OE integrity.

Poster 43

Chao Xue, MS

Program Year: 2013

Advisor: Bradford Berk, MD

Cyclophilin A (CypA) is a Pathogenic Mediator of Pulmonary Arterial Hypertension

Chao Xue[1,2], Mark Sowden[2], Amy Mohan[2], Bradford Berk[1,2]

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Rationale: Pulmonary arterial hypertension (PAH) is a devastating disease in which oxidative stress has been proposed to mediate pathological changes to the pulmonary vasculature such as endothelial cell (EC) apoptosis, endothelial to mesenchymal transition (EndMT), vascular smooth muscle cell (VSMC) proliferation, and inflammation. Our previous study showed that cyclophilin A (CypA) was secreted from EC and VSMC in response to oxidative stress, and much of the secreted CypA was acetylated (AcK-CypA). Furthermore, CypA was increased in the plasma of patients with PAH.

Objective: To evaluate the cell-specific role of CypA in PAH and compare the relative effects of AcK-CypA and CypA on EC apoptosis, development of an inflammatory EC phenotype and EndMT.

Methods and Results: Transgenic overexpression of CypA in EC, but not SMC, caused a PAH phenotype including increased pulmonary artery pressure, α-smooth muscle actin expression in small arteries, and CD45 positive cells in the lungs. Mechanistic analysis using cultured mouse lung microvascular EC showed that CypA and AcK-CypA increased apoptosis measured by caspase 3 cleavage and TUNEL staining. MM284, a specific inhibitor of extracellular CypA, prevented EC apoptosis. In addition, CypA and AcK-CypA promoted an EC inflammatory phenotype assessed by increased VCAM1 and ICAM1 expression, phosphorylation of p65, and degradation of IkB. Furthermore, CypA and AcK-CypA promoted EndMT assayed by change in cell morphology, increased mesenchymal markers and EndMT related transcription factors. At all concentrations, AcK-CypA stimulated greater increases in apoptosis, inflammation and EndMT than CypA.

Conclusions: EC-derived CypA (especially AcK-CypA) causes PAH by a presumptive mechanism involving increased EC apoptosis, inflammation and EndMT. Our results suggest that inhibiting extracellular secreted CypA is a novel therapeutic approach for PAH.

Poster 44

Haiqing Bai, MS

Program Year: 2012

Advisor: David Dean, PhD

Non-transport Functions of the Na^+, K^+-ATPase β1 subunit Contribute to Tight Junction Formation

Haiqing Bai[1], Mike Barravecchia[2], David Dean[2]

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Aim: Acute respiratory distress syndrome (ARDS) is a severe lung disease without effective pharmacologic treatment. Gene therapy may provide a potential cure for this devastating disease. Previous work in our lab has shown that gene transfer of Na/K-ATPase β1 subunit to the lung improves lung function and decreases animal death. Na/K-ATPase is a ubiquitously expressed ion pump that primarily functions to drive the fluid clearance from the alveoli. Unexpectedly, gene transfer of the Na/K-ATPase β1 subunit not only accelerate resolution of lung edema, but also increases the expression of tight junction proteins, which provides additional benefit to reduce lung injury. In order to advance our gene therapy approach towards clinical use, we must understand the molecular mechanism by which the β1 subunit regulates junctional proteins. **Method**: Alveolar type II cells (ATII) were isolated from rats using an IgG-panning approach; alveolar type I cells (ATI) were transdifferentiated from ATII. The protein and mRNA levels of tight junctions were measured by qPCR and Western Blot, respectively. Immunofluorescence staining was used to detect tight junction formation. Transepithelial electrical resistance (TEER) and FITC-dextron were employed to measure epithelial permeability. Interacting partners of the β1 subunit were pulldown, purified by SDS-PAGE, trypsinized and subjected to LC-MS-MS analysis. **Result:** 1) Transfection of β1 subunit induces upregulation of tight junction proteins occludin and zo1 in ATI cells, but not in ATII cells. Increased tight junction proteins in ATI cells also leads to higher TEER and lower permeability. 2) β1 subunit overexpression induces ERK phosphorylation. Inhibiting ERK activation blocks the overexpression of zo1 but not occludin, implying that distinct pathways might be responsible for different junction proteins. 3)Many undescribed protein interactions of the β1 subunit are identified, including GEFH1 and MRCKα, two top hits that belong to the Rho family of GTPase, which has been shown to play important role in tight junction regulation. These results suggest Na/K-ATPase β1 subunit has unique non-transport functions in promoting lung epithelial tight junction formation.

Poster 45

Diana Agostini-Vulaj, DO

Resident: Chief Resident, PGY 3

A Rare Cause of Gastrointestinal Bleeding - Blue Rubber Bleb Nevus Syndrome

Diana Agostini-Vulaj, Chad Cornish, Wenqing Cao

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Blue rubber bleb nevus syndrome (BRBNS) is a rare congenital disorder typically presenting with multifocal venous malformations usually involving the skin and gastrointestinal tract. We report the oldest case of BRBNS diagnosed in a male at 97 years of age. Prior to admission, the patient reported up to two melenic stools per day requiring multiple transfusions with packed red blood cells. Outside video capsule endoscopy was reviewed. An anterograde double balloon enteroscopy was performed followed by repeat video capsule endoscopy which revealed dark blue to purple mucosal bowel protuberances in the jejunum, some with active bleeding (Figure A). A small bowel surgical resection was performed with approximately 90 centimeters of jejunum removed. Pathologic examination revealed multiple blue-purple submucosal lesions up to 2.5 centimeters as well as white-yellow submucosal nodules up to 0.6 centimeters (Figure B). Histologically, these constituted submucosal and transmural hemorrhage associated with abnormal blood vessels as well as lymphatics (Figure C and D). The main differential diagnosis for multifocal vascular malformation includes congenital diseases with vascular malformations such as BRBNS, Hereditary Hemorrhagic Telangiectasia and Klippel-Trenaunay Syndrome. A diagnosis of BRBNS was made based on the endoscopic and pathologic findings and lack of skin lesions for other entities. The prognosis for BRBNS is generally favorable. While most reported cases are sporadic, an autosomal dominant inheritance pattern has been identified on chromosome 9p which encodes tyrosine kinase TIE-2. This entity represents an uncommon cause of gastrointestinal bleeding, which pathologists should consider when presented with multifocal vascular malformations.

Poster 46

Diana Agostini-Vulaj, DO

Resident: Chief Resident, PGY 3

Management of Concomitant Factor VII Deficiency and Factor V Leiden Mutation; Case report

Diana Agostini-Vulaj, Charles W. Francis, Majed A. Refaai

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Factor V Leiden (FVL) mutation is the most common inheritable thrombophilia. Factor VII (FVII) deficiency is a rare bleeding disorder (1/500,000). We report a case of a 58 year-old male diagnosed with both of these entities with no previous medical history. The patient presented to our emergency department with persistent and increasing abdominal pain. CT scan revealed extensive occlusive thrombosis in the main, left, and right portal veins, splenic vein, superior mesenteric vein and its branches. Heparin drip was started (1,750 U/hr). Thrombolysis with tPA was initiated (12.5mL/hr/4 days) with concurrent SC heparin (5,000U/TID). Following successful thrombolysis, enoxaparin (100mg SC) was started with bridge to warfarin (5mg). However, on follow up imaging re-thrombosis was perceived 7 days following the initial presentation. Another cycle of heparin/tPA was started. INR at that point was found to be elevated (5.7). Hemoperitoneum was then detected and anticoagulation therapy was held. Further investigation revealed FVII level of 7% (normal:66-159%) with no inhibitor pattern. A dose of 2,384 U (21.6 U/Kg) of Kcentra was administered and an acceptable level of FVII was achieved and maintained over the next day. A series of small doses of NovoSeven (0.1-1mg) were then started to maintain acceptable levels of FVII over 12 days (Figure). Further workup revealed FVL heterozygous mutation. The patient was discharged on enoxaparin without reoccurrence of bleeding or thrombosis. This case demonstrates the narrow therapeutic window in a patient with both hypercoaguable and hypocoagulable states and successful treatment of a life threatening bleed using both Kcentra and NovoSeven

Poster 47

Diana Agostini-Vulaj, DO

Resident: Chief Resident, PGY 3

Distinction Between Telangiectatic/Inflammatory Adenoma and Mass Effect on Liver Sampling

Diana Agostini-Vulaj, Jennifer J. Findeis-Hosey, Raul S. Gonzalez

University of Rochester Medical Center, Department of Pathology and Laboratory Medicine, Rochester, NY

Context: Telangiectatic/inflammatory hepatocellular adenoma (TIA) is characterized by sinusoidal dilation, inflammation, and occasionally a bile ductular reaction. However, these changes can also be seen in non-neoplastic liver tissue adjacent to a mass lesion. This differential may arise in biopsy tissue attempting to sample a liver mass, and the distinction is crucial in situations such as distinguishing adenoma from unsampled metastatic disease, both of which may present as multiple liver lesions. Serum amyloid A (SAA) immunostaining is useful for the diagnosis of TIA but is not entirely specific. Additionally, the histologic pattern of mass effect (ME) has received little formal scrutiny, and SAA has not been evaluated in this context. To help resolve this differential, this study compares the morphologic and immunohistochemical findings in TIA and ME.

Design: Forty-eight cases were retrieved from our departmental archives, including 36 examples of ME and 12 TIAs. Several histologic findings were evaluated in all cases. SAA staining was performed in cases with available blocks and was scored using previously published criteria.

Results: Strong SAA immunostaining was observed in 100% of TIAs and 58% of ME cases ($P=0.0177$). Histopathologic findings that significantly differed between TIA and ME included ductular reaction ($P=0.0238$), cholestasis ($P=0.0432$), and unpaired arteries ($P<0.0001$) (Table).

Conclusions: Unpaired arteries and strong SAA staining best distinguish TIA from ME. However, the former may be absent due to sampling, and the latter is not available in all laboratories. Cholestasis may also help suggest the diagnosis of TIA, while ductular reaction may suggest ME.

Poster 48

Diana Agostini-Vulaj, DO

Resident: Chief Resident, PGY 3

Implementation of a Pathology-Driven Lynch Syndrome Screening Program

Diana Agostini-Vulaj, Kelly Q. McMahon, Rebecca Amorese, Jennifer J. Findeis-Hosey

University of Rochester Medical Center, Department of Pathology and Laboratory Medicine, Rochester, NY

Background: Lynch Syndrome (LS) is a genetic syndrome driven by germline mutations in mismatch repair (MMR) protein complexes which result in an increased risk for developing numerous neoplasms including colorectal carcinoma (CRC). Multiple laboratory methodologies can be used for the screening and confirmation of LS, including immunohistochemistry (IHC), molecular, and germline mutation analysis. As pathologists are intimately associated with the LS screening process, we are presented with the opportunity to develop comprehensive LS screening programs that go beyond the reporting of IHC and laboratory results. This study outlines the steps taken at an academic hospital to create a comprehensive LS screening program spearheaded by pathologists.

Design: Since May 2012 we have performed MMR IHC with MHL1, PMS2, MSH2, and MSH6 antibodies on all resected CRCs, with reflex BRAF V600E mutation analysis beginning in July 2013. We divided our process into three phases: Phase 1 involved MMR IHC testing without further follow-up or action by pathology; Phase 2 began with the implementation of a monthly Pathology-Genetic Correlative Conference (PGCC) to review all abnormal cases, providing written/verbal follow-up to the providing physician, and implementation of reflex BRAF testing; Phase 3 involved the addition of presenting cases with abnormal MMR IHC staining at our weekly GI Tumor Board (GITB) meeting. The number of abnormal MMR IHC cases subsequently referred to our genetic counseling service (GCS) was examined.

Results: In Phase 1 we demonstrated abnormal MMR IHC staining in 11 (12.5%) of 88 CRCs; follow-up to our GCS was unknown for this phase. In Phase 2, 32 (12.6%) of 254 CRCs had abnormal MMR IHC profiles requiring clinical follow-up; two (6.3%) of these cases were referred to our GCS. In Phase 3, 14 (8.6%) of 166 CRCs had clinically actionable abnormal MMR IHC profiles; three (21.4%) of these cases were subsequently referred to our GCS.

Conclusion: Through molecular and immunohistochemical testing, pathology plays a critical role in the LS screening process. We face the challenge of ensuring that our results are appropriately acted upon by our clinical counterparts. Through the step-wise implementation of a monthly PGCC and presentation of possible LS cases at GITBs we have been able to improve the referral of possible LS patients to our GCS, although there remains room for improvement.

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