2018 Pathology Research Day

Keynote Lecture

Andrew L. Folpe, MD

Phosphaturic Mesenchymal Tumors~~

What I Have Learned

Dr. Folpe attended Amherst College (Amherst, MA) as an undergraduate, the University of Rochester (Rochester, NY) as a medical student, and performed his residency in Anatomic Pathology at the University of Washington Medical Center (Seattle, WA). Dr. Folpe received additional fellowship training in Immunohistochemistry, under the direction of Dr. Allen Gown, and in Soft Tissue Pathology, under the direction of Dr. Sharon Weiss.

Dr. Folpe is the author of over 200 medical publications, principally in the areas of soft tissue pathology and diagnostic immunohistochemistry, the co-author of the 6th edition of Enzinger and Weiss' Soft Tissue Tumors and the most recent Armed Forces Institute in Diagnostic Pathology Series and a member of the consensus conferences for the 3rd and 4th editions of the WHO Classification of Tumors of Soft Tissue and Bone.

Pathology Research Day
Monday, June 11, 2018

Schedule of Events

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

8:00 ~ 8:30	**Continental Breakfast Flaum Atrium**
8:45	**Welcome: Class of '62 Auditorium** **Dr. Bruce Smoller, MD, Chair, Department of Pathology and Laboratory Medicine**
9:00 ~ 10:00	**Oral Presentations Pathology Residents ~**
	Nisha Patel, DO, Resident Microarray CGH-SNP Analysis Detects Frequent Chromosomal Abnormalities Indicating Clonal Cytopenia(s) in Patients With Indeterminate Bone Marrow Dysplasia - An Institutional Study Of 94 Cases
	Alexandra Danakas, DO, Resident Real Time Cytopathology Feedback (RTCF) versus traditional Rapid On-Site Evaluation (ROSE) for Endobronchial Ultrasound Guided Fine-Needle Aspiration (EBUS-FNA) of mediastinal lymph nodes (MLN)
	Anna Israel, MD, Resident NKX3.1 Expression in Salivary Gland neoplasms- Marker for Mucinous Differentiation and Diagnostic Pitfall?
	Mushal Noor, MBBS, Resident Unexpectedly High Prevalence of Cystoisospora belli in Acalculous Gallbladders of Younger Patients
10:15 ~11:15	**Juried Poster Session 1**
11:30 ~12:30	**Juried Poster Session 2** **Flaum Atrium** **Pathology Residents** **Cell Biology of Disease PhD Program in Pathology Students**
11:15 ~ 12:30	**Boxed Lunch Atrium ~~ Conference Attendees**
12:45 ~ 2:00	**Keynote Address:**
	Andrew Folpe, MD Phosphaturic Mesenchymal Tumors ~ What I Have Learned

2:00 ~ 3:00 **Oral Presentations—PhD Students**

Richard Bell, MS, PhD Class of 2014
Genetic Ablation of iNOS in TNF-Tg Mice with Inflammatory-Erosive Arthritis Prevents Lymph Node Expansion and Decreases Synovial Infiltrates

Andrea Amitrano, PhD Class of 2016
Optogenetic Regulation of T Cell Metabolism in the Tumor Microenvironment

Madison Doolittle, MS, PhD Class of 2015
Investigating *Zbt40* as a Determinant of Osteoblast Function and Commitment

Olivia Marola, PhD Class of 2016
Endothelin Signaling in Glaucomatous Neurodegeneration

3:00 ~ 3:30 **Break ~ Coffee and Cookies ~ Atrium**

3:30 ~ 4:30 **Oral Presentations: Residents and Fellows**

Phoenix Bell, MD, Resident
Significance of Clinicopathologic Parameters, Including Margin Distance and Tumor Budding, on Local Disease Recurrence Following Esophageal Endoscopic Mucosal Resection

Numbereye Numbere, MBBS, Resident
Should Ki67 Immunohistochemistry Be Performed on All Lesions in Multifocal Small Intestinal Neuroendocrine Tumors?

Chad A. Hudson, MD, PhD, Fellow
Clinical utility of classical and non-classical monocyte percentage in the diagnosis of Chronic Myelomonocytic Leukemia

Jason Shen, MD, PhD, Fellow
Quantitative measurement of Human Epidermal growth factor Receptor-2 (HER2) protein expression in 'classical' and 'non-classical' FISH categories: a comparative study

4:45 **PhD commencement Awards~ Dr. Richard Libby, PhD, Program Director**

Closing Remarks:
Dr. Bruce Smoller, MD, Chair, Department of Pathology and Laboratory Medicine

5:00 ~ 6:30 **Hors d'oeuvres Reception Flaum Atrium**
Catered by Gatherings

Poster: 1

Nisha Patel, DO

Resident: PGY-4

An overlapping spectrum between TACRD and VACTERL syndromes: no longer two distinct entities?

Nisha Patel[1], Stephanie Laniewski[2], and Philip J. Katzman[1]

[1]Department of Pathology and Laboratory Medicine University of Rochester Medical Center, Rochester, NY, USA, [2]Department of Obstetrics and Gynecology, University of Rochester Medical Center, Rochester, NY, USA

TACRD (tracheal agenesis/atresia, cardiac abnormalities, radial ray defects, and duodenal atresia) syndrome and VACTERL (vertebral defects, anal atresia, cardiac defects, tracheoesophageal fistula, renal anomalies, and limb abnormalities) syndrome are rare conditions characterized by multi-organ malformations that are considered to be two distinct entities. To our knowledge, only three cases in the literature have been reported to have overlapping abnormalities consistent with both syndromes. We report an autopsy case of a preterm 26 week male fetal death in utero in which a tracheoesophageal fistula with esophageal atresia, duodenal atresia, left radial aplasia, pre-axial left first digit hypoplasia, bilateral anomalous pulmonary lobation without isomerism, polysplenia, and Meckel's diverticulum were identified at autopsy. The tracheoesophageal fistula and radial and thumb hypoplasia fit into a VACTERL syndrome, while duodenal atresia, polysplenia, Meckel's diverticulum, and lung hypoplasia are associated with TACRD syndrome. This case appears to best fit into an overlap diagnosis between VACTERL and TACRD. Our findings support the notion that a spectrum exists between these two entities. Increased recognition of closely-related malformations will help better understand embryologic development and heighten our awareness of these findings during post-mortem autopsies.

Poster: 2

Numbereye Numbere, MBBS

Resident: PGY-1

Should Ki67 Immunohistochemistry Be Performed on All Lesions in Multifocal Small Intestinal Neuroendocrine Tumors?

Numbereye Numbere[1], MBBS, Aaron Huber, DO[1], Chanjuan Shi, MD, PhD[2], Justin M. M. Cates, MD, PhD[2], Raul S. Gonzalez, MD[1]

[1]Department of Pathology and Laboratory Medicine, University of Rochester Medical Center, NY, USA , [2]Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, TN, USA

Introduction

Well-differentiated small intestinal neuroendocrine tumors (SI-NETs) are often multifocal, and this has been suggested to impart worse disease-free survival. Practice guidelines have not been established for World Health Organization (WHO) grading of multiple primary lesions – including whether all lesions should undergo immunohistochemical staining for Ki67. In the absence of a scientifically supported approach, most pathologists likely stain the single largest lesion. This study evaluates the link between SI-NET multifocality and other clinicopathologic features, and the link between SI-NET size, multifocality, and Ki67 index.

Materials & Methods

We identified 68 patients with ileal or jejunal SI-NET, who had a combined total of 207 primary lesions. Each case was evaluated for patient age and sex; size of all tumors; presence of lymph node metastases, mesenteric tumor deposits, distant metastases and disease-specific outcome. Ki67 immunohistochemical staining was performed on all 207 primary lesions, and a proliferation index was manually counted from one photographed hot-spot per tumor. The relationship between focality and clinicopathologic factors was compared using Fisher's exact test. Outcome was tested using Cox regression analysis.

Results

Among the 68 patients, 27 had multifocal disease (median 5 lesions, range 2-32), 25 of whom only had WHO grade 1 tumors. For the other two patients, one had two subcentimeter grade 2 lesions (including the largest) and 8 subcentimeter grade 1 lesions, and the other had one 1.6 cm grade 3 lesion and 1 subcentimeter grade 1 lesion.

Most tumors were WHO grade 1 (201/207, 97%), five were grade 2, and 1 was grade 3 (Figure 1). Only three patients with unifocal disease had a grade 2/3 tumor. There was a positive correlation between Ki67 index and tumor size (coefficient 0.28; 95% confidence interval 0.05-0.52, P=0.017) (Figure 2).

Male patients were more likely to have multifocal disease (P=0.047), but patient age was unrelated to multifocality (P=0.97). There was no significant association between disease focality and nodal metastases (P=0.19), metastatic tumor deposits (P=1.0), distant metastases (P=0.43), progression-free survival (P=0.69) or overall survival (P=0.30). Adjuvant treatment had no effect on overall survival (P=0.12). Patient receiving adjuvant treatment had worse progression-free survival (P=0.001), likely due to higher disease burden beforehand.

Conclusion

In patients with multifocal SI-NET, unless a particular lesion has a high mitotic rate, only staining the largest lesion for Ki67 should serve to accurately grade essentially all cases. This approach is likely already followed at most institutions.

SI-NET multifocality does not appear to impact patient survival.

Reference

Yantiss RK, Odze RD, Farraye FA, Rosenberg AE. Solitary versus multiple carcinoid tumors of the ileum: a clinical and pathologic review of 68 cases. Am J Surg Pathol. 2003;27:811-817.

Poster: 3

Mushal Noor, MBBS

Resident: PGY-2

Unexpectedly High Prevalence of Cystoisospora belli in Acalculous Gallbladders of Younger Patients.

Mushal Noor MD[1], Philip J. Katzman MD[1], Christa Whitney-Miller MD[1], Jennifer Findeis-Hosey MD[1], Aaron R. Huber DO[1], Raul S. Gonzalez MD[1], Z. David Zhou MD PhD[1], Henriette D. N'kodia*1, Kathryn Skonick*1, Rebecca L. Abell DO2, Lawrence J. Saubermann MD [2,3], Laura W. Lamps MD[4], Michael G. Drage MD PhD [1]

[1]Departments of Pathology, [2]Pediatrics , and [3]Gastroenterology and Hepatology, University of Rochester Medical Center, Rochester, NY USA. [4]Department of Pathology, University of Michigan, Ann Arbor, MI USA.

Background:
A recent review of the NY State Planning and Research Cooperative System Longitudinal Administrative Database (spanning 1995-2013) revealed that indications for cholecystectomy have changed dramatically. Calculous cholecystitis has declined (-20% p < 0 .0001), while other indications increased: acalculous cholecystitis (+94%; p < 0 .0001), biliary dyskinesia (331.74%; p < 0 .0001), and biliary colic (+55%; p=0.0013). There has been a concomitant shift toward operating on a younger patient population. The etiology for these changes in the clinical context and patient population undergoing cholecystectomy remains unknown.
Given the recently reported association of Cystoisospora belli (Cb) infection with acalculous disease of young, we undertook a single institution retrospective review of cholecystectomies lacking stones by gross examination in patients less than 30 years of age.

Design:
Archival slides from 219 cholecystectomies without gallstones were reviewed, 29 were excluded due to autolysis of greater than 50% of the biliary epithelium. 190 well-preserved cholecystectomies without gallstones were scored for the presence/absence of parasitophorous vacuoles characteristic of Cb.
Location of the vacuoles (cystic duct vs other) was recorded. Correlation of the presence of Cb with patient factors was determined by Fisher Exact Test.

Results:
The 190-patient cohort comprised 136 females and 54 males (mean age 18.8 yrs; range < 1 to 29). Of the entire cohort, 19 (10%) were positive for Cb infection, ranging in age from 7 to 29 years of age. Of the 54 males, 10 (18.5%) were positive for Cb; of the 136 females, 9 (6.6%) were positive. Cb infection was positively associated with male sex (p = 0.028).

Conclusion:
Cb infection is more prevalent amongst immunocompetent humans than previously recognized. Further studies are warranted to determine whether the presence of Cb in acalculous gallbladder disease represents an etiologic agent, or a consequence of factors predisposing to acalculous gallbladder disease.

Poster: 4

Caroline Bsirini, MD

Resident: PGY-3

Liver Histology in Septic Patients: Is It All About Ductular Cholestasis?

Caroline Bsirini, Raul Gonzalez

University of Rochester Medical Center

Sepsis often causes cholestatic jaundice, and liver biopsy may be performed to exclude other diagnoses. Cholestasis within bile ductules is generally touted as a key histologic finding in the liver of septic patients. However, it is not always present, nor is it entirely specific. Additionally, the spectrum of other histopathologic findings in septic patients has not been thoroughly studied.For126 liver biopsies where sepsis was mentioned in the provided clinical information or in the pathologic differential diagnosis, we searched medical records for patient outcome, clinical impression (sepsis or not), blood culture results, and whether processes that might cause overlapping histologic changes (e.g., total parenteral nutrition or large duct obstruction) were present. We evaluated each case for histologic findings, including portal and lobular inflammation, ductular reaction, duct injury, lobular or ductular cholestasis, and acidophil bodies. Histologic findings between patients with and without clinical sepsis, and between patients with Gram-positive vs. Gram-negative results on blood culture, were compared using Fisher's exact test. Common histologic findings in clinically septic patients (n=79) included portal chronic inflammation (55cases, 70%), lobular acute inflammation (46, 58%), ductular reaction (60, 76%), lobular cholestasis (69, 87%), ductular cholestasis (53, 67%), and acidophil bodies (37, 47%), though 19 patients (24%) had other diagnoses with potential histologic overlap. Findings between clinically septic and non-septic patients were similar, though the latter more often had lobular chronic inflammation (22% vs. 40%, P=0.027). Ductular cholestasis rates were similar in both groups (67% vs. 53%, P=0.13). There were no significant differences among findings in patients with Gram-positive vs. Gram-negative sepsis, though the former tended to have acidophil bodies more often (64% vs. 32%, P=0.069). Clinically septic patients more often died soon thereafter than clinically non-septic patients (P=0.0002), lending credence to that categorization. Ductular cholestasis can be present in septic and non-septic liver samples, though its presence should at least suggest the possibility of sepsis. Other common findings in sepsis include lobular cholestasis, ductular reaction, portal chronic inflammation, lobular acute inflammation, and acidophil bodies. Clinical history should always be reviewed for potentially confounding cholestatic conditions.

Poster: 5

Joseph Blitman, MB, BCh, BAO

Resident: PGY-3

Is the Rate of Frozen Section Discordance Affected by Subspecialty Sign Out?

Joseph H. Blitman, MB, BCh, BAO, Brandon Buscaglia, Christa L. Whitney-Miller, MD, David G. Hicks, MD, Aaron Huber, DO

University of Rochester

Background: Monitoring frozen section (FS) and final permanent section (PS) correlation is a valuable quality assurance metric in surgical pathology. Discordant FS and PS results may alter clinical management. In July 2015, our department implemented full subspecialty sign out (SSSO) while maintaining general sign out of frozen sections. The discordant FSs, at our institution, are categorized as minor if there is little or no perceived or actual clinical significance and major if there is major or potentially major clinical significance, which is determined by the final sign out pathologist. We sought to determine if the SSSO model has adversely impacted our FS and PS discordance rate.

Design: We retrospectively evaluated the discrepancy rates (DRs) before (January 2012-June 2015) and after (July 2015-December 2017) SSSO. The DRs were compared for the minor, major and combined disagreements (minor + major) before and after SSSO using the student's t-test.

Results: There were 7,045 total frozen sections with 4,056 prior to SSSO and 2,989 after SSSO of which 139 had minor disagreements (74 prior to SSSO and 65 after SSSO)and 42 had major disagreements (26 prior to SSSO and 16 after SSSO). The average combined DRs pre and post SSSO were 2.17 and 3.0, respectively. The difference was statistically significant for the minor (p=0.005), not statistically significant for the major (p=1), and statistically significant for the combined (p=0.014) disagreements.

Conclusions: The data shows that SSSO, at this institution, appears to increase FS discrepancy rates (minor and combined disagreements). This suggests that when adopting a SSSO model, maintaining competency with a wide array of specimens seen on a general intraoperative consultation service may be challenging and requires careful monitoring of frozen and permanent section discrepancy rates.

Poster: 6

Jian Shen, MD, PhD

Fellow: Breast Pathology

Quantitative measurement of Human Epidermal growth factor Receptor-2 (HER2) protein expression in 'classical' and 'non-classical' FISH categories: a comparative study.

Jian Shen, Brandon Buscaglia, Hideki Goda, Loralee McMahon, Takako Natori, Bradley Turner, Hisatake Okada, Yasushi Nakano and David G. Hicks.

Targeting HER2 protein overexpression in breast cancer has been shown to be an effective therapeutic modality. One methodology of assessing HER2-status is fluorescent in situ hybridization (FISH). FISH evaluates HER2 gene amplification, which is a surrogate for protein expression. FISH results are classified based on the HER2/CEP17 ratio and HER2 gene copy number. FISH relies on the assumption that the HER2 gene copy numbers accurately reflect the amount of protein that is translated in tumor cells. In the current study, we use a novel immunodetection methodology utilizing streptavidin coated Phosphor-integrated dot fluorescent nanoparticles (PID) to quantitatively measure HER2 protein expression in different FISH categories.

159 cases of invasive breast cancers, which had previously undergone HER2 FISH testing, were selected for this study. Cases were sorted and categorized into 'classical' (groups 1 and 7) and 'non-classical' (groups 2-6) FISH categories (Figure 1). PID testing was performed on all cases, and the PID HER2 protein expression was compared to HER2 FISH results by category.

Both 'classical' FISH categories correlated, as would be expected, with HER2 protein expression (Figures 1 and 2). However, 'non-classical' FISH categories were found to have very low-levels of HER2 protein expression, except for polysomy ratio positive cases (group 2), which had similar protein expression to 'classical' FISH amplified cases (Figures 1 and 2) ($P < 0.001$).

Our results show that HER2 protein expression in four out of five 'non-classical' FISH categories (groups 3-6) were all comparable to the 'classical' non-amplified FISH category when measured by PID. This suggests that these 'non-classical' FISH categories may be less likely to respond to targeted HER2 therapy. Furthermore, HER2 protein expression in group 2 was comparable to the 'classical' amplified FISH category when measured by PID. This suggests that this 'non-classical' FISH category may be more likely to respond to targeted HER2 therapy. The findings of this study show that neither HER2/CEP17 ratio, nor HER2 gene copy number alone can accurately predict the HER2 protein expression in all cases. The correlations between PID HER2 protein expression and FISH categories suggests that quantification of HER2 protein with PID will add value in determining HER2 status for targeted HER2 therapy. Follow up studies with a larger patient cohort are warranted.

Figure 1: Flow diagram of different FISH categories and corresponding PID results

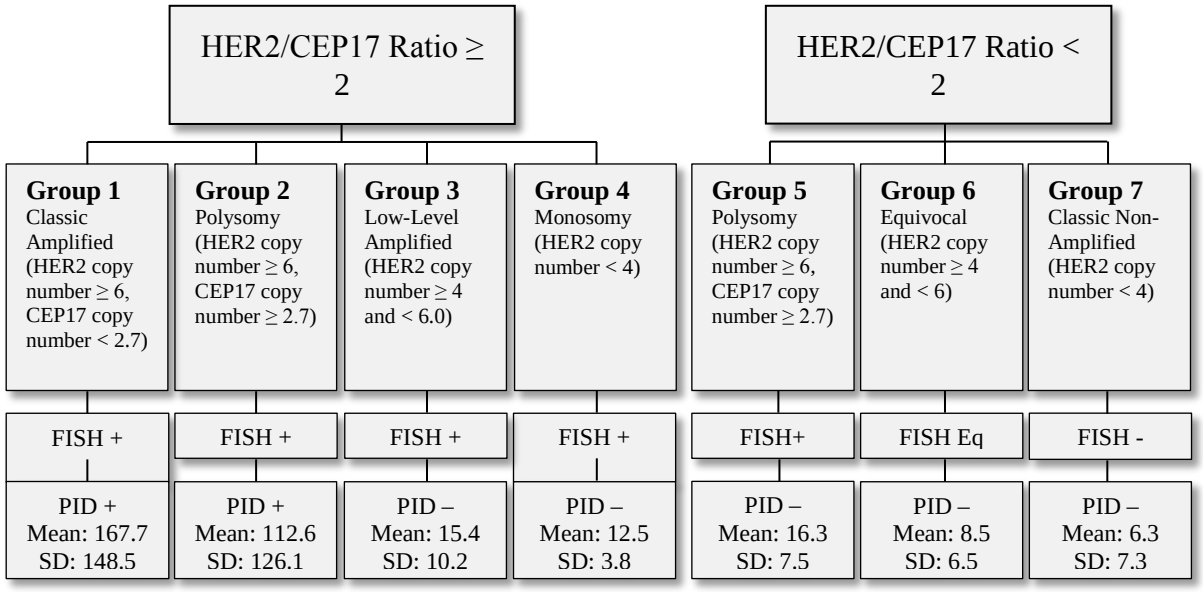
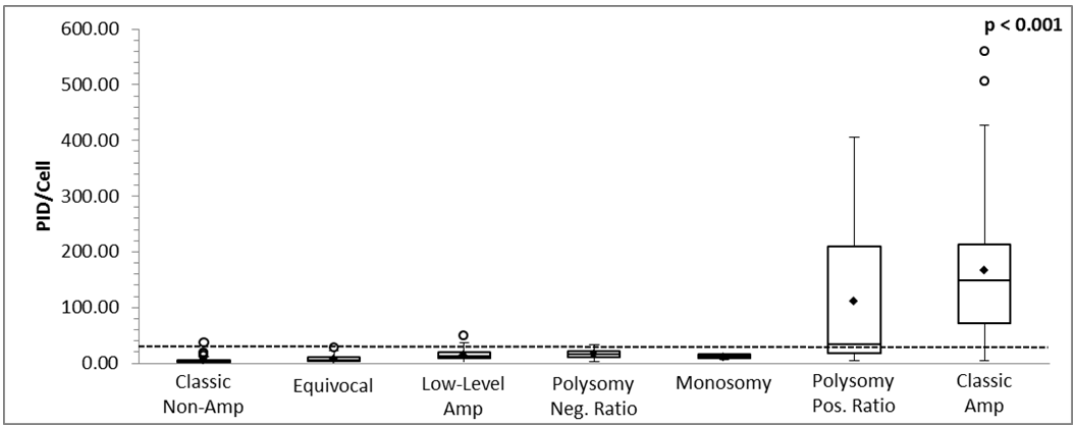
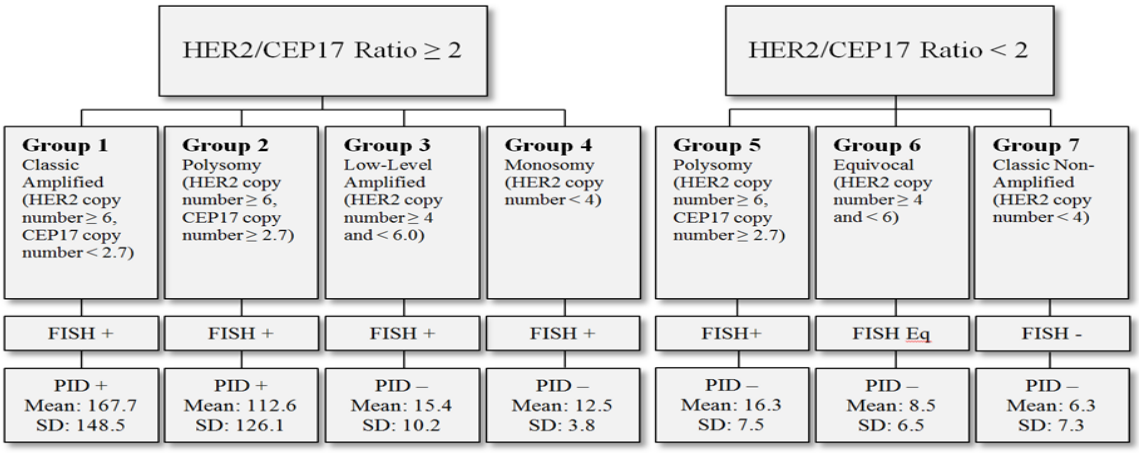


Figure 2: Correlation of FISH HER2 categories HER2 protein overexpression measured by IHC-PIDs



Picture form of Figure 1:



Poster: 7

Meenal Sharma, MBBS

Fellow: GU Pathology

Clinical Significance of Perivesical Lymph Node Metastasis in Radical Cystectomy for Bladder Cancer

Meenal Sharma, MBBS; Jerome Jean-Gilles Jr, MD; Hiroshi Miyamoto, MD, PhD

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

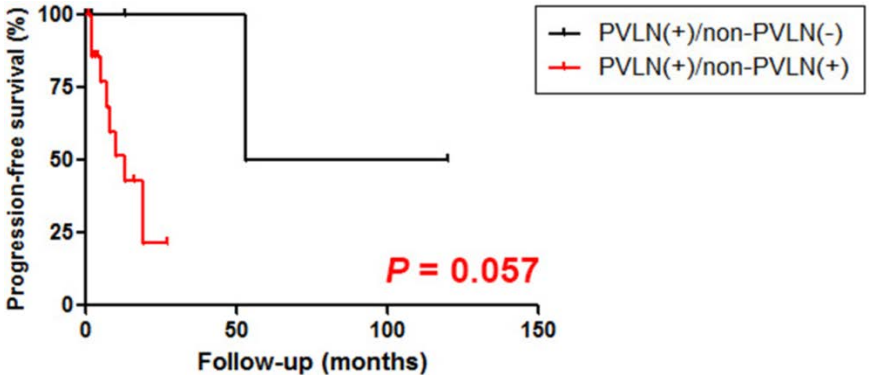
Background:
It is well documented that pelvic lymph node (LN) metastases in bladder cancer are associated with a poor prognosis. Perivesical LNs (PVLNs) are occasionally isolated in the fat around the bladder during grossing of cystectomy specimens and can be involved in the primary lymphatic drainage.Little is known about the prognostic implications of the involvement of PVLNs. AJCC 8th edition stages positive PVLN(s) as N1 or N2 disease category

Study design:
We searched our Surgical Pathology database (July 2004 to January 2018) and found 111 radical cystectomy cases where PVLNs were isolated (mean: 2.3; median 2; range: 1-14).For analysis, the cases were divided into following four groups:

Group 1: PVLN(-)/non-PVLN(-)
Group 2: PVLN(+)/non-PVLN(-)
Group 3: PVLN(-)/non-PVLN(+)
Group 4: PVLN(+)/non-PVLN(+)

Results:

- Patients with PVLN(-)/non-PVLN(+) or PVLN(+)/non-PVLN(+) disease, but not PVLN(+)/non-PVLN(-) disease, had significantly worse prognosis than those with PVLN(-)/non-PVLN(-) disease.
- Patients with PVLN(+)/non-PVLN(-) disease tended to have a lower risk of disease progression (P = 0.057), compared to those with PVLN(+)/non-PVLN(+) concurrent positive pelvic LN.



Conclusions:

- While Lymph nodes metastasis is associated with aggressive disease, slightly better outcomes in patients with isolated positive PVLN than in those with concurrent positive pelvic LN following radical cystectomy are implied.

- While pathologic analysis of PVLNs in cystectomy specimens is important, our findings may not readily support the AJCC 8th edition staging system where PVLN metastasis is staged as pN1 or pN2.

Poster: 8

Roula Katerji, MD

Resident: PGY-2

Concurrent polycythemia of undetermined Etiology and plasma cell myeloma

Roula Katerji, MD, Chad A. Hudson, MD, PhD

Department of Pathology, University of Rochester Medical Center, Rochester, NY

Cases with mutated immunoglobulin variable gene regions have a better prognosis, as does CLL/SLL with 13q deletion. Trisomy 12 and a normal karyotype are associated with an intermediate prognosis. In contrast del 11q, del 17p, and del 6q are associated with a poorer prognosis. CLL/SLL with ZAP-70 or CD38 expression is associated with an unmutated status and, therefore, has a more aggressive course and adverse prognosis. ZAP-70 and CD38 have been used as surrogate markers for immunoglobulin gene mutational status.

Discussion: The etiology of the polycythemia is undetermined, particularly in light of the varying EPO levels (normal at presentation, now markedly elevated).

As a trademark manifestation of myeloma is anemia, it is very uncommon for polycythemia and myeloma to coexist, particularly when the polycythemia is not due to polycythemia vera (5 cases over 70 years (Hutchison et al., 2016)).

There is data indicating that treatment of the myeloma by Bortezomib in such cases can lead to a concomitant decrease in the hematocrit. Whether this is due to is a mechanistic link between the myeloma and polycythemia or Bortezomib having separate, direct effects on the polycythemia is unknown.

Poster: 9

Anna-Karoline Isreal, MD

Resident: PGY-1

NKX3.1 expression in salivary gland neoplasms- marker for mucinous differentiation and diagnostic pitfall?

Anna-Karoline Israel, MD and Abberly Lott Limbach, M.D.

University of Rochester

Intro: NKX3.1 plays an important rule in prostate development and proliferation and is currently used as a diagnostic biomarker for prostate cancer. Expression of NKX3.1 has been reported in salivary gland tissue and submucosal bronchial glands, but not in salivary gland neoplasms. In our study, we examine the expression of NKX3.1 in select salivary gland neoplasms.

M&M: The pathology laboratory information system was searched and 38 cases salivary gland neoplasms (diagnoses included: pleomorphic adenoma, warthin's tumor, acinic cell carninoma, adenoid cystic carcinoma, oncocytoma, mucoepidermoid carcinoma, salivary duct carcinoma, epithelial-myoepithelial carcinoma and polymorphous low-grade adenocarcinoma) were identified. Immunohistochemical staining for NKX3.1 was performed and any amount and any intensity of nuclear staining was considered a positive staining. The number of tumors with positive staining as well as the number of cases with staining in the background normal gland was recorded.

Results: There were 38 salivary neoplasms from 17 male and 21 female patients (age range of 20-94 years, average 60.7 years) examined in this study. The cases included both benign and malignant tumors, see table 1. We observed strong positive staining in one case of acinic cell carcinoma with high grade transformation. Additionally, positive staining was seen in mucoepidermoid carcinoma, salivary duct carcinoma, epithelial-myoepithelial carcinoma, pleomorphic adenoma, and warthin tumor, see table 1. There were 8 of cases with strong positivity in the background mucous glands of the submandibular and minor salivary glands.

Conclusions: In this study we assessed NKX3.1 expression in 38 representative cases of salivary gland neoplasms. Positivity for NKX3.1 may be suggestive of high grade transformation of acinic cell carcinoma and salivary duct carcinoma, but may represent low-grade stage in mucoepidermoid carcinoma. Mucinous glands in the submandibular gland and minor salivary glands show positive NKX3.1 staining and thus may represent a diagnostic pitfall when assessing primary salivary neoplasms and metastatic disease. Further studies are needed to assess the full potential of NKX3.1 staining in salivary neoplasms.

Tumor Type (n)	NKX3.1 Positive in tumor	NKX3.1 Positive in background gland
Pleomorphic adenoma (5)	2	2
Warthin tumor (5)	5	0
Oncocytoma (5)	0	0
Acinic cell carcinoma (5)	3	1
Adenoid cystic carcinoma (5)	3	2
Mucoepidermoid carcinoma (6)	3	2
Salivary duct carcinoma (3)	2	0
Epithelial-myoepithelial carcinoma (3)	2	0
Polymorphous low-grade aenocarcinoma (1)	1	1

Poster: 10

Chia-Hao Wu, MS

Program Year: 2015

Advisor and Department: Yi-Fen Lee, PhD, Urology

Extracellular vesicles derived from malignant and non-malignant cell origins play an opposite role in tumorigenesis.

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

[1]Departments of Cell Biology of Disease, and [2]Urology, University of Rochester, Rochester, New York, USA.

Introduction and objectives: Extracellular vesicles (EVs) are released by most cell types including cancer cells. EVs carry cargos of protein, nucleic acid and lipid and serve as important cell-cell communication mediators. EVs' role in tumorigenesis is indefinite, which seems to depend on their cargo and cell of origin. To better delineate EVs' role, this study focuses on comparing EVs derived from malignant or non-malignant cell origins of their impact on causing stress or malignantly transforming the recipient cells.

Methods: EVs from TCC-SUP, a bladder cancer cell line, and SV-HUC, an immortalized urothelial cell line, were collected and purified. SV-HUC cells were used as recipients. Molecular alterations in EV-treated cells were assayed by Western blot. Production of reactive oxygen species (ROS) were compared by DCFDA assay. Tumorigenicity was determined by an *in vitro* colony formation assay and an *in vivo* xenograft mouse model.

Results: Bladder cancer EVs increase, while non-malignant urothelial EVs decrease, ER stress signaling sensor protein PERK and IRE1 expression, and the two EV groups elevate different levels of ROS in the SV-HUC recipient cells. Long-term cancer EV treatment leads to up-regulated tumorigenesis *in vivo* and *in vitro*, in contrast to a reduced level of *in vitro* colony formation followed by a long-term non-malignant urothelial EV treatment. Mass spectrometry and miRNA microarray revealed a different cargo composition in these vesicles.

Conclusions: Our data reveal EVs from cancer or non-malignant origins play opposite roles in tumorigenesis. Cancer EVs increase cellular stress, which may accelerate predisposed recipient cell evolution toward malignancy, and thus lead to an upregulated tumorigenesis. The non-malignant EVs, on the contrary, mitigate the ER stress signals and colony formation in the SV-HUC cell. This study provides a mechanism by which the bladder cancer EVs can promote tumorigenesis, and, for the first time, shows that non-malignant urothelial EVs are protective against malignant transformation.

Poster: 11

Ronghao Wang, MS

Program Year: 2014

Advisor and Department: Chawnshang Chang, PhD, Department of Pathology

A trans-splicing event of AR transcript in prostate cancer cells

Ronghao Wang[1], Yin Sun[1], Chawnshang Chang[1,2]

[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
[2]Sex Hormone Research Center, China Medical University and Hospital, Taichung, 404, Taiwan

Prostate cancer (PCa) is the second leading cause of cancer-related death among men in western countries. Therefore, dissection of the underlying mechanisms and developing the new drugs to fight against PCa have important implications. It is widely accepted that androgen receptor (AR) signaling plays crucial role in the initiation and progression of PCa. As a nuclear receptor, AR recognizes its responsive DNA element (5'-GGA/TACANNNTGTTCT-3',) and transcriptionally regulates a broad range of genes including PSA, FKBP5 and TMPRSS2 upon androgen stimulation, providing survival signals to PCa cells. Since AR activity mainly requires ligand binding, androgen deprivation therapy (ADT) has become the popular treatment for PCa, which effectively cures PCa patients for 2-3 years before the development of castration resistant prostate cancer (CRPC). 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. Nevertheless, Enz resistance caused by multiple mechanisms limits its further application.

In previous studies, we already found that AR-v7, an AR variant without ligand binding domain, was induced by *Malat1* (metastasis associated lung adenocarcinoma transcript 1) in C4-2 enzalutamide-resistant cells to cause resistance. In addition, we also uncovered a new AR variant (named as AR-v33 due to its duplicated exon3) dramatically induced in our established Enz-resistant PCa cells based on divergent PCR analysis. Sequence analysis using two-round PCR revealed that this AR variant has intact AR's exons but with additional exon3. This AR variant could interact with AR-v7 to render enzalutamide resistance to PCa and deficiency of its expression could restore Enz sensitivity in EnzR cells. Importantly, our preliminary data also 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. Compared to cis-splicing that works on single pre-mRNA, trans-splicing occurs when two independent pre-mRNAs are fused together to form a chimeric RNA which encodes a novel protein. Although the process of trans-splicing is very rare in vertebrates, its occurrence may be involved in the numerous physiological and pathological processes including cancer progression. We hypothesize that this new AR variant plays roles in prostate cancer progression. In the future, we would like to uncover the biological functions of this new AR variant and to find which trans-splicing related signaling molecules responsible for its production. Also, the biological contributions of the extra exon3, which encodes zinc-finger domain, to AR-v33 deserves our intensive investigation.

Poster: 12

Xiaoting Ma, MS

Program Year: 2013

Advisor and Department: Stephen Hammes, MD, PhD, Department of Medicine, Division of Endocrinology and Metabolism

Paxillin Mediates Genomic Proliferative Signals in Prostate Cancer

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

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Paxillin, best known as a focal adhesion associated adaptor protein, is extensively involved in focal adhesion signaling and kinase signaling throughout the plasma membrane and cytoplasm. However, recent studies suggest that paxillin also plays a critical role in regulating gene expression within the nucleus. In prostate cancer cells, paxillin serves as a critical liaison between cytoplasmic and nuclear signaling, mediating not only androgen or growth factor induced extranuclear MAPK signaling, but also MAPK- and Androgen Receptor (AR)-induced intranuclear gene transcription. In fact, paxillin is overexpressed in human prostate cancer tumor microarrays, suggesting that it may serve as an important biomarker for prostate cancer. Here, we utilize an RNA-Seq strategy to take a global view of the paxillin transcriptome in prostate cancer cells. RNA-seq data from PC3 cells with reduced paxillin expression reveals that paxillin activates several pro-proliferative pathways, including the CyclinD/Rb/E2F and DNA replication/repair pathways. Paxillin also downregulates several pro-apoptotic genes including *CASP1* and *TNFSF10*. However, functional studies in prostate cancer cell lines indicate that paxillin primarily promotes cell proliferation by induction of cell cycle progression, and paxillin shows minimum effects on prostate cancer cell apoptosis. Overexpression of paxillin in the prostatic epithelial cell line RWPE-1 confirms that paxillin promotes cell proliferation and upregulates cell cycle related gene expression. Additionally, knocking down paxillin in dihydrotestosterone (DHT) treated LNCaP cells eliminates approximately 1000 androgen responsive genes, some of which are signature genes involved in endocrine therapy resistance. Thus, in prostate cancer, paxillin appears to increase cell proliferation, induce cell cycle progression, enhance androgen responsive gene transcription, and promote hormone therapy resistance. Paxillin might therefore serve as a therapeutic target for both androgen-sensitive and castration resistant prostate cancer.

Poster: 13

Fu-Ju Chou, MS

Program Year: 2014

Advisor and Department: Chawnshang Chang (CBD)

Radiation therapy-induced androgen receptor splicing variant ARv7 decreases the subsequent 2nd line ADT-Enzalutamide therapy efficacy

Fu-Ju Chou[1], Hao Tian, Yuhchyau Chen[2], Don Chen[1], Shuyuan Yeh[1], Yuanjie Niu[1], and Chawnshang Chang[1]

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Radiotherapy(RT) uses high-energy rays or particles to kill cancer cells. For early stage of prostate cancer(PCa) patients, RT can provide similar therapeutic efficacy as prostatectomy. Nowadays more and more advanced prostate cancer (PCa) patients choose RT as primary therapy, however, the potential risk and side effects that impact the subsequent 2nd line androgen-deprivation-therapy (ADT) with antiandrogen enzalutamide (Enz) remains unclear.

Here we found RT could change the mRNA splicing profile and further increase the expression of androgen receptor splicing variant 7 (ARv7), the key factor for the induction of Enz resistance. The clinical evidence from advanced PCa patients' circulating tumor cells (CTCs) exhibit higher expression level of ARv7 after RT. Mechanism dissection revealed that RT might induce the lncRNA-Malat1 expression and further alternatively splice out ARv7, which might then promote PCa reprogramming to cancer stem cells to increase Enz resistance. Here, we use multiple PCa cell lines to prove that combined RT with malat1 inhibitor or AR/ARv7 degradation enhancers including Cisplatin (or Carboplatin) or ASC-J9® could restore the Enz-sensitivity for the subsequent 2nd line ADT-Enz treatment, and can better suppress the advanced PCa cell growth.

Together, these findings not only discover the potential risk and unwanted side effect of RT also provides a novel and clinically available options as adjuvant therapies for RT to better suppress the advanced PCa progression.

Poster: 14

Carlos Ortiz-Bonilla, MS

Program Year: 2015

Advisor and Department: Yi-Fen Lee, PhD, Urology

BCG internalization by bladder cancer cells increases their secretion levels of extracellular vesicles

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Introduction: Intravesical Bacillus Calmette-Guérin (BCG) immunotherapy has been used to treat non-muscle invasive bladder cancer (BC) for 40 years but its underlying mechanism remains largely unknown. It is known that BCG adheres to integrin $\alpha5\beta1$integrins in the urothelial lining, which promotes its internalization through macropinocytosis and triggers an immune response cascade. Extracellular vesicles (EVs), small membrane-bound vesicles, act as immune modulators by transferring molecular cargos to recipient cells. We hypothesize that BCG internalization by BC cells increases their immune active EV release which could play key roles in mediating BCG-induced anti-tumor host immune responses, so the patient derived EVs may serve as predictive biomarkers that can differentiate BCG responders from non-responders.

Methods: BC cell lines, human T24, 674V, HT1197, J82, RT4 and 5637 were treated with 1- $4x10^6$ CFU/ml live TICE® BCG. After 4-72 hours, BCG internalization was assessed by PCR analysis. Cell lysate, total RNA and EVs were collected for immuno-molecular profiling by quantitative PCR and Western blotting analyses. In addition, secreted EVs were isolated by serial ultracentrifugation and analyzed by Nanoparticle Tracking Analysis (NTA). The differences in BCG internalization capacity, alterations in gene expression and EVs release in response to BCG were compared.

Results: In response to BCG treatment, RT4 cells showed low BCG internalization levels and no changes in their EV secretion rates. Their immuno-molecule expressions at cellular level were not significantly altered after BCG treatment. Also, J82 cells showed low BCG internalization level. However, their EV secretion rate was significantly altered after BCG treatment. In contrast, T24, 674V, HT1197 and 5637 BC cell lines showed evident BCG internalization and their EV secretion rates were significantly increased after BCG treatment. In addition, the expression of the key molecules in modulating immune response, such as MHC and co-stimulatory molecules, were significantly upregulated as a result of BCG treatment.

Conclusions: We conclude that BCG treatment resulted in increased EV release from BC cells with evident BCG internalization levels. In addition, BCG induced expression of key immuno-modulatory molecules in those BC cells. However, this effect was not seen in cells with low BCG internalization capacity. All these results together suggest that the increased EV release by some BC cells after BCG treatment seems to rely on their BCG internalization capacity. Therefore, changes in EVs detected in patients' urine during BCG immunotherapy can be further explored as predictive biomarkers.

Poster: 15

Andrea Amitrano

Program Year: 2016

Advisor and Department: Minsoo Kim, PhD, Department of Microbiology and Immunology

Optogenetic Regulation of T Cell Metabolism in the Tumor Microenvironment

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The tumor microenvironment presents significant metabolic challenges to T cells by depleting oxygen, glucose, and other key metabolites. Therefore, T cells and tumor cells engage in fierce metabolic competition which promotes T cell exhaustion. Exhausted T cells cannot carry out effector functions and are incapable of killing cancer cells. To overcome the metabolite deficiency of the tumor microenvironment and boost anti-tumor effector functions in exhausted T cells, we developed a genetically encoded light-activated proton pump (fungal proton pump, “Mac”), namely photoactivatable oxidative phosphorylation (PA-OxPhos) that is expressed in the inner mitochondrial membrane. During OxPhos, electrons enter the electron transport chain (ETC), causing protons to be pumped across the inner mitochondrial membrane to establish a proton gradient. This gradient is then used to generate ATP through complex V. We hypothesize that the outward proton pumping across the inner mitochondrial membrane by light stimulation of PA-OxPhos will mimic the ETC function and boost ATP generation in T cells, even with low levels of metabolites. This approach can provide T cells a competitive metabolic advantage in the tumor microenvironment. PA-OxPhos can be expressed in the mitochondria of transfected 293T cells, HeLa cells, and activated mouse $CD8^+$ T cells. Light stimulation of 293T cells expressing PA-OxPhos successfully increased ATP production even in the presence of 2-deoxy-D-glucose (2-DG), a glucose analog that inhibits glycolysis. Our data suggests that PA-OxPhos can remotely provide a competitive metabolic advantage and hypothetically boost T cell functions in the tumor microenvironment. The utilization of an alternative mechanism for ATP production in T cells could potentially dissipate the failures of current T-cell-based cancer immunotherapies in destroying malignant cells of solid tumors.

Poster: 16

Chad A. Hudson, MD, PhD

Fellow: Hematopathology

Increased AID-Generated Acquired Glycosylation Sites in Diffuse Large B-cell Lymphomas with IGH-BCL2 and CD10 Expression

Chad A Hudson, Janice Spence, Diana Adlowitz, Madalynn Bryant, W Richard Burack

University of Rochester Medical Center

Background:
B cell development is substantially shaped by the enzyme activation-induced deaminase (AID) which regulates two major steps in B-cell development, one of which is somatic hypermutation (SHM), the production of mutations in the variable regions of immunoglobulin genes. AID is induced in germinal center B cells and is highly expressed in germinal center B cell neoplasms. We have previously shown that in follicular lymphoma (FL), a germinal center B-cell-derived lymphoma, the immunoglobulin heavy chain gene contains more AID/SHM-generated acquired glycosylation sites in the variable region (IGHV) than IGH from marginal zone lymphoma. Diffuse large B cell lymphoma, the most common mature B cell neoplasm, is often separated into two groups by cell of origin, germinal center-derived and non-germinal center-derived. Two important markers for germinal center origin are the presence of an IGH-BCL2 translocation and CD10 expression. We hypothesize that like FL, germinal center-derived GCBs will have an increased predilection for harboring acquired IGHV glycosylation sites.

Design:
The clonally rearranged IGH gene in 32 DLBCL 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:
The 32 DLBCL cases were categorized by IGH-BCL2 translocation status (13 positive/17 negative/2 not classified), CD10 expression (25 positive/7 negative), and cell of origin by the Hans' algorithm (27 germinal-center B-cell type (GCB) and 5 activated B-cell type (ABC)). Twelve of thirteen IGH-BCL2-positive DLBCL cases had an acquired glycosylation site vs 8 of 17 IGH-BCL2-negative cases ($p=0.02$). Similarly, CD10-positive cases were more likely to have an acquired glycosylation site than CD10-negative (20/25 vs 1/7, $p=0.003$) as were GCB cases vs ABC (20/27 vs 1/5, $p=0.037$). There were no significant differences in SHM rate between groups regardless of categorization method.

Conclusion:
Both the presence of an IGH-BCL2 translocation and CD10-positivity, markers of germinal center origin, are associated with an increased likelihood to have an acquired glycosylation site. These data suggest that acquired glycosylation sites in IGHV may contribute to the distinctive biology of t(14;18)-positive and CD10-positive DLBCL.

Poster: 17

Chad Hudson, MD, PhD

Fellow: Hematopahology

Lack of MUM1 Expression Characterizes B-Lymphoblastic Leukemia/Lymphoma

Chad A Hudson, Roula Katerji, W Richard Burack

University of Rochester Medical Center

Background:
While the differential diagnosis of B-lymphoblastic leukemia/lymphoma (B-LBL) versus an aggressive mature B-cell neoplasm is usually not challenging, there are cases in which this is a diagnostic dilemma. In such cases, the immunophenotype of the large malignant blast-like cells is often “in between” the normal lymphoblast phenotype (CD10/CD19/CD34/TdT-positive, surface light chain-negative) and a mature B cell phenotype (CD19/surface light chain-positive) and there is a relative lack of additional immunostains that can be used to differentiate between the two entities. MUM1 (IRF4) is a transcription factor that in B cell lymphomas is often used as a marker of post-germinal center cell of origin. As the cell of origin in B-LBL is not a post-germinal B cell, MUM1 is an intriguing candidate as a marker that would favor against B-LBL in these challenging cases.

Design:
MUM1 expression was determined by immunohistochemistry in 30 cases of B-LBL and 55 cases of aggressive B-cell neoplasms (48 cases of diffuse large B cell lymphoma, 6 cases of high-grade B cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements).

Results:
Twenty-nine of the 30 B-LBL specimens were negative for MUM1 expression with the one positive case showing dim, variable expression. On the other hand, 59% (33/54) of the aggressive mature B cell neoplasm cases were positive for MUM1 expression, indicating that as expected, B-LBL specimens were significantly less likely to be MUM1-positive ($p<0.001$). Further, when only adult cases of B-LBL were considered (the population in which this diagnostic dilemma is most relevant), MUM1 was uniformly negative (0/18).

Conclusion:
These data indicate that the evaluation of MUM1 expression is useful when the differential diagnosis includes B-LBL as well as other aggressive B-cell neoplasms.

Poster: 18

Chad A. Hudson, MD, PhD

Fellow: Hematopathology

AID-Generated Acquired IGH Glycosylation Sites but Not Somatic Hypermutation Rate Differentiate Low-grade versus High-grade Follicular Lymphoma

Chad A Hudson, Janice Spence, Diana Adlowitz, W Richard Burack

University of Rochester Medical Center

Background:
B cell development is substantially shaped by the enzyme activation-induced deaminase (AID) which functions include regulating the production of mutations in the immunoglobulin genes (somatic hypermutation (SHM)). AID is induced in germinal center B cells and is highly expressed in follicular lymphoma (FL), a neoplasm of germinal center B cells. FL-derived IGH is more likely to have AID/SHM-generated acquired glycosylation sites (AG sites) in the variable region (IGHV) than IGH from both non-neoplastic B cells and the malignant B cells in non-germinal center-derived B cells neoplasms, making glycosylation site status an intriguing marker for FL. This proclivity for IGHV glycosylation in FL is thought to be related to antigen-independent signaling mediated by glycosylated immunoglobulin. As it is thought that signaling pathways supporting centrocytes and centroblasts differ, we sought to test if IGHV glycosylation is different in centrocyte-rich (low grade, grade 1-2) and centroblast-rich (high grade, grade 3) FL.

Design:
The clonally rearranged IGH gene in 41 low-grade FL specimens and 11 high-grade FL 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/SHM-generated AG sites were determined by analysis of the predicted protein sequence.

Results:
Low-grade FL had significantly more AG sites per specimen than high-grade FL (1.38±0.98 vs 0.73±0.47, p=0.037). Further, while there was no significant difference between low-grade and high-grade FL in having at least one AG site (36/41 vs 8/11, p=0.3), there was a significant difference in having multiple AG sites as 30% of low-grade FL specimens (12/41) had multiple AG sites while none (0/11) of the high-grade FL specimens had multiple AG sites (p=0.05). There was no difference in SHM rate between low-grade and high-grade FL (low-grade: median: 87% identity to germline sequence, range: 69-96%; high-grade: median: 86%, range: 83-92%).

Conclusion:
While AG sites are a general feature of FL, a low-grade FL specimen is significantly more likely to have multiple AG sites in the IGHV region than a high-grade FL specimen. This suggests that the accumulation of multiple AG sites might be a feature unique to low-grade FL and may be a useful factor in differentiating low-grade FL vs high-grade FL in diagnostically challenging cases.

Poster: 19

Sohaib Abu-Farskh, MD

Resident: PGY-4

Interobserver agreement in the diagnosis of anal dysplasia

Sohaib Abu-Farsakh, M.D., Michael Drage, M.D., Aaron Huber, M.D., Bradley Turner, M.D., Sharlin Varghese, M.D., Xi Wang, M.D., Christa Whitney-Miller, M.D., Raul S. Gonzalez, M.D.

University of Rochester Medical Center

Introduction: Management of anal dysplasia relies on the accurate diagnosis of anal tissue biopsy and anal cytology specimens, as low-grade squamous intraepithelial lesion (LSIL) is generally managed with observation, while high-grade squamous intraepithelial lesion (HSIL) often requires ablation. Previous studies have shown that anal dysplasia can be subjective, with significant interobserver variability, despite existing histologic criteria. As institutions move toward subspecialty signout (SSSO), decisions must be made regarding whether to assign anal biopsies to the gastrointestinal (GI) or gynecologic (GYN) pathology service. We investigated interobserver agreement in the diagnosis of anal dysplasia, comparing GI and GYN pathologists.

Methods: We identified 200 tissue biopsies of anal mucosa and circulated them along three GI pathologists and three GYN pathologists. Each pathologist separately scored each biopsy as normal, atypical, LSIL, or HSIL. The GI pathologists then met to establish a consensus diagnosis on the cases with discordant individual interpretations. The GYN pathologists also held a consensus meeting. Weighted kappa coefficients were calculated to reflect the agreement between each GI pathologist and the GI consensus diagnoses, each GYN pathologist and the GYN consensus diagnoses, and the GI with the GYN consensus diagnoses.

Results: The GI pathologists agreed diagnostically on 97 (49%) cases prior to consensus; the GYN pathologists agreed on 33 (17%). Weighted kappa coefficients for the agreement between each GI pathologist and the GI consensus diagnoses ranged from 0.529 to 0.668; for the GYN pathologists with the GYN consensus diagnoses, they ranged from 0.104 to 0.719. The weighted kappa coefficient for the agreement between GI and GYN consensus diagnoses was 0.633. The GI pathologists diagnosed 14 cases as HSIL, with four (29%) agreed upon prior to consensus. The GYN pathologists diagnosed 14 cases as HSIL, with 13 (93%) agreed upon prior to consensus; 11 were called HSIL by both groups.

Conclusions: In general, interobserver agreement on the diagnosis of anal dysplasia was moderate to good, but significant variability was still seen. In our study, the GI pathologists had a tighter range of interobserver variability, but the GYN pathologists had more consistent individual interpretations of HSIL. Institutions with SSSO will likely need to weigh their own individual practice characteristics in determining whether to assign anal biopsies to GI or GYN pathologists.

Poster: 20

Sohaib Abu-Farskh, MD

Resident: PGY-4

Clinicopathologic features of incidental meningiomas found at autopsy

Sohaib Abu-Farsakh, M.D., Mahlon Johnson, M.D., Ph.D.

University of Rochester Medical Center

Introduction:
Meningiomas are the most common primary brain tumors. The features of meningiomas removed by neurosurgical procedures are well characterized; however, very few reports focused on the features of incidental meningiomas found at autopsy. In this study, we compared the clinicopathologic features of incidental meningiomas found at autopsy with those of meningiomas removed by surgery.

Metarials and methods:
We searched our archives for the past 12 years for meningiomas found incidentally during autopsies. Cases that were diagnosed before death were excluded. We compared the age, gender, tumor location, histological type and histological grade between autopsy meningioma cases and 76 recent surgical meningioma cases.

Results:
Fourteen out of the 27 cases of incidental meningioma were in male patients (male: female ratio of 1.1 : 1), compared to 21 out of the 76 surgical meningioma cases (male: female ratio 0.38 : 1). The average age for autopsy meningioma cases was 66.7 compared to 57.8 for surgical cases. A difference was noted in the frequency of histological types, notably, incidental autopsy meningiomas were more likely to be of psammomatous type (6/27 cases) compared to surgical meningiomas (7/76 cases). Tewnty-six out of the 27 incidental meningiomas were grade 1 tumors with only one grade 2 tumor found, in contrast to 24 grade 2 and two grade 3 meningiomas found among the 76 surgical cases. Of the 27 incidental meningiomas, 20 were located in the cerebral convexities, 6 were near the skull base, and one patient had multiple lesions. For the 76 surgical meningiomas the tumor location was as follows: 46 in the cerebral convexities, 21 in the brain stem/skull base, and 9 in the spinal cord.

Conclusions:
Incidental meningiomas found at autopsy are more likely to be grade 1 meningiomas and to be of psammomatous type.

Poster: 21

Diana Agostini-Vulaj, DO

Fellow: GI Pathology

Incidence and Significance of GATA-3 positivity in Pancreatic Ductal Adenocarcinoma and cholangiocarcinoma

Diana Agostini-Vulaj, DO[1], Laura E. Bratton, MD[3], Richard F. Dunne, MD[2], Justin M. Cates, MD, PhD[4], Zhongren Zhou, B.Med, PhD[5], Jennifer J. Findeis-Hosey, MD[1], Qi Yang, AAS[1], Mira K. Ramesh, HSD[1], Raul S. Gonzalez, MD[6]

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Context: GATA-3 is a reliable immunohistochemical marker for mammary and urothelial carcinoma. It is reportedly positive in 10-37% of pancreatic ductal adenocarcinomas (PDACs) and 3-9% of cholangiocarcinomas (CCs). GATA-3 positivity in these tumors has not been analyzed relative to clinical or histologic findings. We aimed to determine whether GATA-3 positivity in PDACs and CCs is associated with pertinent clinicopathologic features.

Design: Slides from tissue microarrays containing 240 PDACs and 60 CCs were stained using a monoclonal antibody against GATA-3. Percentage and staining intensity of GATA-3-positive tumor nuclei were recorded. Clinicopathologic parameters evaluated included patient age, sex, race, and overall survival; (neo)adjuvant therapy; lymphovascular and/or perineural invasion; and tumor site, size, grade, histologic subtype, and pathologic stage. Margin status was also analyzed for PDAC.

Results: Positive staining for GATA-3 was seen in 38 of 240 (16%) PDACs and 3 of 60 (5%) CCs. GATA-3 positivity in PDAC cases was more likely in male patients (P=0.013) and in tumors with perineural invasion (P=0.011). GATA-3 positive PDACs trended toward worse survival on multivariate analysis (P=0.074). The only 3 GATA-3-positive CCs were poorly differentiated (P=0.069); low case number precluded multivariate survival analysis for CCs.

Conclusions: GATA-3 staining is uncommon in PDACs and CCs, consistent with previous reports. This positivity appears to have little relevance to patient outcome. The fact that these tumors can occasionally express GATA-3 should be considered when interpreting immunohistochemical results from a metastatic lesion of unknown primary, in order to avoid misdiagnosis.

Poster: 22

Diana Agostini-Vulaj, DO

Fellow: GI Pathology

Extent of Lesional Cell Spread in Hepatic Epithelioid Hemangioendothelioma: Implications for the Diagnosis in Minimal Samples

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Departments of Pathology, [1]University of Rochester Medical Center, Rochester, NY; [2]Emory University, Atlanta, GA; [3]University of Florida, Gainesville, FL; [4]Brigham and Women's Hospital, Boston, MA; [5]Vanderbilt University, Nashville, TN; [6]Formerly at Medical College of Wisconsin, Milwaukee, WI; [7]Beth Israel Deaconess Mcedical Center, Boston, MA.

Background: Epithelioid hemangioendothelioma (EHE) can arise in the liver and typically has a WWTR1-CAMTA1 fusion. Lesional cells are known to involve the sinusoids of adjacent parenchyma, but this phenomenon has received little scrutiny. Following an index case in which an attempt to biopsy a mass lesion (ultimately diagnosed as EHE) yielded only adjacent parenchyma with lesional cells in sinusoids, we undertook this study to further characterize these cells.

Design: We identified 18 cases of hepatic EHE (including the index case), and a comparison group of 6 EHEs from other sites. All had classic EHE cytomorphology, lacking high-grade cytologic atypia. For all cases, we recorded EHE multifocality and largest lesion size. We identified lesional cells away from the main tumor mass (when present) and noted their location, maximum distance from the main tumor, density per high-power field (hpf), and cytomorphology. Immunohistochemical staining for CAMTA1, ERG, and CAM 5.2 was performed on all cases.

Results: Lesional cells were present away from the main mass in 17 of 18 (94%) liver cases, always within sinusoids and occasionally (4/17, 24%) in central veins. They were intensely hyperchromatic with vaguely cerebriform nuclei; they appeared multinucleated in 6 (35%) cases, somewhat mimicking megakaryocytes. They were positive for CAMTA1 and ERG in all 17 cases, though ERG also stained sinusoidal endothelium. Two cases (12%) were focally positive for CAM 5.2. The number of cells per hpf ranged from 12 to 80 (mean 32). The main EHE lesion was available for comparison for 16 of the 17 cases with lesional cells in sinusoids. Sinusoidal EHE cells ranged from 0.1 to 0.8 cm away from the main tumor (mean 0.35 cm). Fifteen main EHEs contained morphologically similar cells, though they were less dense (range 1-9 cells/hpf, mean 4). There were no statistically significant associations between cell density and EHE size or multifocality. In the 6 non-hepatic cases, tumor cells did not extend beyond the main EHE.

Conclusions: Lesional cells in hepatic EHEs often extend beyond the main lesion into sinusoids, where they demonstrate an unusual and somewhat distinctive morphology. They may be of utility in confirming the diagnosis of EHE, particularly in situations where the main lesion is not present in the sampled tissue or shows unusual morphology. CAMTA1 staining best assists in identifying these cells.

Poster: 23

Diana Agostini-Vulaj, DO

Fellow: GI Patholgy

Significance of Method of Lymph Node Involvement in Pancreatic Ductal Adenocarcinoma

Diana Agostini-Vulaj, DO[1], Justin M. M. Cates, MD, PhD[2], Richard F. Dunne, MD[3], Raul S. Gonzalez, MD[4]

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Background: Pancreatic ductal adenocarcinoma (PDAC) is an aggressive neoplasm with notoriously poor disease-specific survival. Lymph node (LN) metastases are associated with adverse outcome. Unlike some other organs, peripancreatic LNs are anatomically situated very close to the head of the pancreas, allowing PDAC to sometimes involve nodes by direct extension rather than by lymphovascular invasion (LVI). To our knowledge, only one previous study has evaluated whether method of LN involvement impacted survival, finding no difference. This study aimed to examine the mechanism of LN involvement in PDACs further and to assess their clinicopathologic relevance. Size of tumor foci within LNs was also compared to outcome.

Design: For 264 PDAC resections from 1998-2017, we evaluated patient age, sex, race, and disease-specific survival, and tumor site, size, grade, stage, margin status, LVI, perineural invasion, LN involvement, and size of largest nodal disease focus. LNs were further characterized as being involved either by direct extension (dir-LN) or lymphohematogenous spread (met-LN). Cases associated with intraductal papillary mucinous neoplasm or mucinous cystic neoplasm were excluded. Associations between method of LN involvement and clinicopathologic factors were assessed using standard bivariate statistical methods. Disease-specific survival by method of LN involvement was compared by Cox proportional hazard regression adjusted for AJCC pM status, adjuvant therapy, age, and tumor size.

Results: The 264 PDACs included 80 cases without LN metastases and 184 with LN metastases. Among the LN-positive cases, 26 were dir-LN only, 90 were met-LN only, and 68 had both met-LN and dir-LN involvement. Increasing tumor size correlated with the number of lymph nodes involved by direct extension (negative binomial regression, incidence rate ratio 1.19, 95% CI 1.02-1.38; P=0.022). Pairwise comparison of coefficient from Cox regression showed no significant differences in log hazard between patients with direct vs. lymphohematogenous LN involvement (HR 1.04; 95% CI 0.51-2.10; P=0.92). Increasing size of the largest focus of nodal involvement did not quite show statistical significance (HR 1.06, 95% CI 0.99-1.14, P=0.089).

Conclusions: LN involvement in PDAC, and the number of nodes with disease, represent important prognostic features with respect to disease recurrence and patient survival and management. With respect to LN involvement mechanisms in PDAC patients, dir-LN and met-LN involvement have similar outcomes. Additionally, the size of nodal metastasis had no prognostic relevance.

Poster: 24

Diana Agostini-Vulaj, DO

Fellow: GI Pathology

Florid Vascular Proliferation of the Colon and Small Bowel: a Potential Sarcomatous Impersonator

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Vascular abnormalities and lesions of the small bowel and colon are rare. A florid vascular proliferation (FVP) associated with colonic obstruction and intussusception has been described and can mimic a vascular tumor including angiosarcoma. We report a case of colonic FVP associated with colonic obstruction and a case of small bowel FVP associated with Meckel diverticulum.

Case 1 was a 62-year-old woman with multiple medical problems and biopsy proven ischemic colitis. She was initially managed conservatively and developed large bowel obstruction with pan-ischemic colitis requiring total colectomy. The resection specimen demonstrated colonic transmural FVP with ulceration highlighted by CD31 immunohistochemical stain. Case 2 was an 80-year-old man with multiple medical problems who presented with four months of abdominal pain. Computed tomography scan demonstrated mesenteric haziness suggestive of a mesenteric mass. Intraoperatively, jejunal diverticulosis and Meckel diverticulum were observed. The resection specimen similarly demonstrated small bowel transmural FVP, as in case 1, with mild endothelial cell atypia and scattered mitoses.

Although the pathogenesis of FVP is not entirely clear, it is thought to represent a benign reactive process. Colonic FVP has been associated with colonic obstruction and intussusception. To our knowledge, FVP has not been reported in the small bowel or in association with Meckel diverticulum. Given the ability to mimic a sarcoma, pathologists should be aware of FVP to avoid this diagnostic pitfall.

Poster: 25

Phoenix Bell, MD

Resident: PGY-1

Significance of Clinicopathologic Parameters, Including Margin Distance and Tumor Budding, on Local Disease Recurrence Following Esophageal Endoscopic Mucosal Resection

Phoenix Bell[1], ILKe Nalbantoglu[2], Justin Cates[3], Raul Gonzalez[1]

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Background*:* Endoscopic mucosal resection (EMR) is widely employed for treatment of esophageal dysplasia and low-stage carcinoma. A detailed, large-cohort analysis evaluating margin status, margin clearance, and tumor budding in EMR specimens has not been performed.

Design: We evaluated 270 esophageal EMR specimens with glandular neoplasia from 133 patients. We recorded patient age, patient sex, specimen fragmentation (i.e., 1 or $\geq$1 tissue fragment per endoscopic lesion), intestinal metaplasia, highest-grade lesion, margin status and clearance, cancer invasion depth, lymphovascular invasion (LVI), and tumor budding (using published guidelines counting one hotspot in a 0.785 mm^2 field). These factors were compared to local disease recurrence, defined as the same or a higher-grade lesion recurring within 2 cm of the prior EMR site.

Results: Adenocarcinoma was the highest-grade lesion in 42% of specimens (39% T1a, 3% T1b), high-grade dysplasia in 36%, and low-grade dysplasia in 22%. Average age at first EMR was 66 years. Seventeen percent of specimens were fragmented. Age had a minor but significant effect on recurrence risk (hazard ratio [HR] 1.02 per year older, 95% confidence interval [CI] 1.00-1.04, P=0.033). Fragmentation did not significantly influence recurrence (P=0.072), nor did "positive tissue edges" in fragmented specimens (P=0.52 for dysplasia, P=0.49 for carcinoma). Positive margins increased recurrence risk in intact (non-fragmented) specimens (P<0.001), but in specimens with negative margins, margin clearance did not significantly influence recurrence (HR=0.71 per millimeter clearance, 95% CI 0.49-1.03, P=0.074). Adenocarcinoma recurrence risk was not affected by tumor budding (P=0.82), LVI (P=0.70), or depth of invasion (P=0.28).

Conclusions*:* Positive resection margins increase the risk of recurrence for intact EMR specimens, but not fragmented ones, which supports labeling margin status "not evaluable" in fragmented specimens. As lesional distance from margin was not significantly associated with risk of recurrence, appropriate minimum clearance cannot be recommended based on these data. Tumor budding and lesional depth do not influence local recurrence in malignant EMRs. These results offer some insight into endoscopic management of Barrett's metaplasia-related lesions, but they may be confounded by "field effect," as subsequent development of dysplasia or malignancy may be due to the abnormal local microenvironment rather than the pathologic characteristics of prior EMR specimens.

Poster: 26

Chao Xue, MS

Program Year: 2013

Advisor and Department: Bradford Berk, MD, Cardiovascular Research Institute

Extracellular Cyclophilin A (CypA) Acts as an Inflammatory Cytokine to Promote Endothelial to Mesenchymal Transition that Contributes to Pulmonary Arterial Hypertension

Chao Xue[1,2], Mark Sowden[2], Sharon Senchanthisai[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) inflammation and endothelial to mesenchymal transition (EndMT). Our previous study showed that a transgenic mouse that overexpressed cyclophilin A (CypA) specifically in EC spontaneously developed PAH associated with inflammation. We also found that acetylated CypA (AcK-CypA) was more potent than CypA in stimulating EC inflammation measured by VCAM1 and ICAM1 expression.

Objective: To compare the relative effects of extracellular CypA and AcK-CypA on EC inflammatory phenotype and EndMT, and explore the signal transduction mechanisms responsible for EndMT.

Methods and Results: MM218, a specific inhibitor of extracellular CypA, prevented EC inflammation induced by CypA and AcK-CypA. Mechanistic analysis using cultured human and mouse pulmonary microvascular EC showed that CypA and AcK-CypA promoted EndMT, assayed by change in cell morphology, decreased EC specific markers, increased mesenchymal markers and EndMT associated transcription factors. In addition, CypA and AcK-CypA caused EC secretion of several EndMT related cytokines such as IL-6. Furthermore, pulmonary microvascular EC isolated from the EC CypA overexpression mice showed an EndMT phenotype compared with those from WT mice.

Conclusions: Extracellular CypA (especially AcK-CypA) causes PAH by a presumptive mechanism involving inflammation and EndMT. The pathways that lead to EndMT provide many targets for new therapies to treat PAH.

Poster: 27

Jessica Ackerman

Program Year: 2017

Advisor and Department: Richard Libby, PhD, Cell Biology of Disease PhD Program in Pathology

Therapeutic potential of a small molecule MRTF-A inhibitor for treatment of lung fibrosis.

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Pulmonary fibrosis is a progressive, destructive lung disease characterized by excessive scarring, resulting in impaired gas exchange and respiratory failure. Although current therapies relieve symptoms, no biological treatments exist to alter the course of the disease. Fibrogenesis is defined as an out of control wound healing response, and myofibroblasts are important for matrix deposition and wound contracture. While these cells are essential to the healing process, their persistence and accumulation at the site of injury is thought to drive fibrosis. MRTF-A, or myocardin related transcription factor A, has been shown to be required for TGF-β induced fibroblast to myofibroblast differentiation in cardiac fibrosis following myocardial infarction, but this has yet to be investigated in the lung. We hypothesized that MRTF-A is also a common driver of fibrosis in the lung, through its ability to drive fibroblast differentiation. We further investigated the anti-fibrotic effects of salinomycin, which has been determined to be a potent inhibitor of TGF-β induced myofibroblast differentiation. Using siRNA, we knocked down MRTF-A expression in primary human lung fibroblasts, and achieved a 30% knockdown in mRNA expression. This reduction in MRTF-A attenuated the morphological changes observed following TGF-β treatment, and cells maintained their original fibroblast phenotype. The myofibroblast marker α-SMA was also significantly reduced with MRTF-A knockdown. MRTF-A overexpression was performed to determine its effects on lung fibroblast differentiation with an adenovirus expressing mouse MRTF-A. Transfected fibroblasts strongly differentiated into myofibroblasts, contracting and expressing high levels of α-SMA. Salinomycin treatment was able to prevent this differentiation and was also sufficient to significantly reduce the levels of all myofibroblast markers tested. Interestingly, baseline levels of MRTF-A were also decreased with salinomycin treatment, demonstrating that salinomycin is a potent small molecule inhibitor of myofibroblast differentiation. and may have therapeutic potential for treatment of pulmonary fibrosis.

Poster: 28

Tiffany Duong

Program Year: 2017

Advisor and Department: Richard Libby, PhD, Cell Biology of Disease PhD Program in Pathology

The Regulation of MRTF-A in the Epicardium by Hypoxia

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Heart failure is defined as the inability of the heart to provide sufficient blood to meet the body's metabolic demands and it is considered to be the leading cause of death worldwide. The "fetal gene program" is reactivated in the epicardium during heart failure but it is not enough to generate repair. Elucidating the mechanisms that control epicardial cell regulation and maintenance in response to physiological cues is important for the treatment of ischemic heart disease. Myocardin-related transcription factors (MRTF) have been shown in preliminary data from the Small lab and published literature to interact with Serum-response factor and Wilms tumor 1 factor in response to physiological hypoxia. The primary goal of this rotation project was to examine the role MRTF-A plays in hypoxia. Performing immunohistochemistry on epicardial cell explants isolated from mouse embryonic hearts revealed a significant increase in the expression of MRTF-A in cells exposed to hypoxic conditions. Additionally, doing co-immunoprecipitation suggested a possibility that MRTF-A is interacting with hypoxia complexes, similar to the HIF pathway. Altogether this preliminary study leads to the conclusion that MRTF-A is a substrate for the hypoxia pathway. Additional studies to fully elucidate MRTF-A function and regulation in epicardial cells could lead to further understanding of the repair and regeneration response in damaged tissue caused by ischemic heart disease. This will aid in the development of treatment methods for heart failure patients.

Poster: 29

Jonathan Bartko, MS

Program Year: 2016

Advisor and Department: Marc Halterman, MD, PhD; Dept. of Neurology, Stroke Division

Contextual Regulation of CHOP-10's Nuclear Localization Sequence

Jonathan Bartko[1,2], Laura Yunes-Medina[2,3], Marc W. Halterman[2,4]

[1]Department of Pathology and Laboratory Medicine, [2]Center for Neurotherapeutics Discovery, [3]Department of Neuroscience, [4]Department of Neurology

In the United States this year, over a half-million people will suffer cardiac arrest. Many of these patients will have neurologic deficits, and a full neurocognitive recovery is often elusive. The transient global ischemia (TGI) experienced during cardiac arrest is characterized by ischemia-induced transcription, which drives delayed neuronal death in selective populations, particularly in the CA1 field of the hippocampus. A clearer understanding of the molecular signaling networks involved in limiting selective neuronal vulnerability may lead to therapies that limit damage and enhance post-ischemic recovery. The viability of this approach is enforced by the *extended window after global ischemic injury to manipulate physiologically responsive transcriptional switches*. The bZIP transcription factor CHOP-10, typically associated with ER-dependent toxicity, contributes to ischemic neuroprotection. While investigating putative mechanisms of CHOP-dependent protection, we identified a novel BDNF-responsive phosphoepitope (Ser107) within the nuclear localization sequence (NLS) that is sufficient to prevent CHOP-dependent toxicity *in vitro*. RNAseq analysis revealed many high-profile, CHOP-regulated genes involved in neuronal survival or apoptosis. We hypothesize that a second NLS site (Thr101) regulates localization to the nucleus. Phosphorylation-regulated NLSs (prNLS), present in some transcription factors, underscores an important mechanism for syncing transcriptional specificity with physiologic responses *in vivo.* Based on these findings *we test the hypothesis that BDNF signaling governs the switch between CHOP-dependent adaptive or pathologic transcription in post-ischemic neurons through effects on prNLS post-translational modification*.

Poster: 30

Shira Winters, MD

Resident: PGY-3

A case series of therapeutic plasmapheresis use in patients with heparin-induced thrombocytopenia undergoing cardiopulmonary bypass with heparin

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University of Rochester Medical Center

Purpose: Evidence on use of therapeutic plasma exchange (TPE) to decrease heparin-PF4 specific antibodies in patients with heparin-induced thrombocytopenia (HIT) who require cardiopulmonary bypass (CPB) with heparin is limited to a few small case series and case reports. We seek to add to available evidence on use of TPE in patients with HIT who require procedures with heparin.

Methods: Between July 2016 and September 2018 three cases were identified in which TPE was used to treat patients with HIT who needed to undergo CPB. We collected demographic, clinical, laboratory, and outcome data on the patients.

Results: Three patients presented requiring surgical intervention. All three received a new diagnosis of HIT and went on to undergo TPE and CPB during the same admission.

The first patient is a 58 year old male who presented with heart failure in the setting of an acute myocardial infarction, requiring initiation of extracorporeal membrane oxygenation (ECMO). Heparin-PF4 antibody was positive, with subsequent recovery of platelet count following heparin cessation. One week later, the patient underwent TPE daily for 4 days in anticipation of CPB to place ventricular assist device (VAD). Heparin-PF4 antibody was negative after three days of TPE. Two weeks later he underwent surgical intervention with heparin without incident. A non-occlusive right brachial artery thrombus was identified at presentation, but was unchanged throughout his admission.

Patient two is a 28 year old male who also developed HIT while on ECMO for heart failure. Heparin-PF4 antibody testing was positive. TPE was performed daily for three days. Heparin-PF4 antibody was negative after day one of TPE. Following completion of TPE, the patient had a VAD placed with intraoperative heparin without incident. No bleeding or thrombotic complications were noted. The patient's platelet count began to recover within 24 hours of TPE completion, despite reexposure to heparin during surgery. Heparin-PF4 antibody three days after VAD placement was negative and remained negative until discharge four weeks later.

The third patient is a 69 year old male who required VAD placement for coronary artery disease. During his admission, he received heparin for atrial fibrillation and developed HIT with positive Heparin-PF4 antibody. In order to undergo VAD placement, he was treated with TPE daily for two days. Heparin-PF4 antibody was negative after one day of TPE. Five days later, he received heparin during VAD placement without bleeding or thrombotic complications. No significant decrease was seen in platelet count following heparin administration during VAD placement.

Conclusions: This case series illustrates that TPE can safely and effectively reduce heparin-PF4 antibodies and allow patients with HIT to tolerate subsequent heparin exposure for necessary surgical procedures. Larger studies are needed to further evaluate TPE use in this population.

Poster: 31

Raman Baldzizhar, MD

Resident: PGY-4

Autopsy case of pulmonary embolism with underlying plasma cell neoplasm

Raman Baldzizhar, Chad A. Hudson, Hani Katerji, Bruce I. Goldman

Multiple myeloma is characterized by the neoplastic proliferation of plasma cells producing a monoclonal immunoglobulin. The diagnosis of MM requires clonal bone marrow plasma cells ≥10 percent or biopsy-proven bony or soft tissue plasmacytoma plus either presence of related organ or tissue impairment or presence of a biomarker associated with near inevitable progression to end-organ damage.

62-year-old black male with past medical history of back pain, obesity, hypertension, and diabetes had a witnessed "falling out" where he fell backwards and became unresponsive. After EMS arrival he was found in pulseless electrical activity. Patient was pronounced soon after arrival to the hospital. The most remarkable gross autopsy findings were bilateral thromboemboli in segmental and subsegmental pulmonary arteries. Microscopically histology of vertebral bone showed hypercellular (90%) for the age bone marrow with majority of the cells being atypical plasma cells. Immunohistochemical stains show cells of interest being positive for CD138, light chain lambda, and immunoglobulin G. Protein electrophoresis of postmortem blood showed presence of M-spike and 1 g/dl paraprotein and serum immunofixation showed IgG Lambda paraprotein in the gamma region.

In conclusion, this report provides evidence that even when cause for sudden death is obvious grossly some routine histology may provide valuable information about underlying disease. In this case myeloma significantly contributed to the fatal embolic event by production of hypercoagulable state. It was observed, that in patients with newly diagnosed and untreated myeloma increases in Von Willebrand factor factor and factor VIII, and a decrease in protein S levels result in a hypercoagulable state which might promote the development of thrombo-embolic complications.

Poster: 32

Raman Baldzizhar, MD

Resident: PGY-4

Autopsy Case of Nephronophthisis and Fat Overload Syndrome

R. Baldzizhar, P. Katzman

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Background
Nephronophthisis is an autosomal recessive, genetically heterogenic renal disease with identified mutations in a number of genes that encode proteins involved in the function of primary cilia. The infantile form is characterized by mutations in the NPHP2 gene, which leads to end stage renal disease. Extrarenal manifestations may include bone anomalies, hepatosplenomegaly and portal fibrosis, situs inversus, septal cardiac defects.

Methods
Patient was a 22 month old female with a past medical history of failure to thrive, chronic kidney disease, nephrogenic diabetes insipidus, G-tube dependence, and chronic liver disease of unclear etiology. Two liver biopsies were most consistent with acute hepatitis and a renal biopsy showed chronic tubulointerstitial nephritis, secondary focal segmental glomerulosclerosis, proliferative arteriolosclerosis, and microscopic renal dysplasia. She was re-admitted with acute worsening of hyperbilirubinemia and transaminitis with electrolyte disturbance. On day 10 of her hospital stay patient inadvertently received IV lipid infusion over 2 hours rather than the typical 20 hours and subsequently developed respiratory distress, increasing abdominal ascites, and bleeding from mucous membranes and intravenous lines sites. Patient was resuscitated with blood products and became more alert with improved respiratory status. Later she had sudden decompensation, received cardiopulmonary resuscitation, more blood products, electrolyte replacement, and abdominal paracentesis. Unfortunately the patient was unable to be resuscitated and died.

Results
Autopsy gross findings included anasarca, multiple small abdominal and parietal petechial hemorrhages. Serosanguineous fluid was found in the pleural cavities and abdominal cavity. Microscopic findings confirmed diagnosis of macronodular liver cirrhosis accompanied by marked cholestasis and bile duct proliferation with intervening areas of parenchymal hemorrhagic necrosis. Pancreas showed acute pancreatitis. Kidneys had bilateral patchy chronic interstitial nephritis with interstitial fibrosis and cortical necrosis with atrophic tubules with dilatation, variably thickened basement membranes, and calcifications, and immature glomeruli.

Conclusion
This case represents an interesting combination of a rare inherited disease and a rare potentially fatal treatment complication. By whole exome sequencing this patient had a novel (non-inherited) STAT3 mutation that was not described in patients with nephronophthisis. The fat overload syndrome described after rapid infusion of lipids can also be accompanied by hepatosplenomegaly, respiratory distress, and spontaneous hemorrhage associated with anemia, leukopenia, thrombocytopenia and coagulopathy. It is not clear in this case to what extent the two diseases overlapped to cause the patient's death. However, given this patient's pre-existing nephronophthisis with declining status, the fat overload syndrome may have been the factor leading to death.

Poster: 33

Brianna H. Shares, MS

Program Year: 2015

Advisor and Department: Roman A. Eliseev, PhD, Center for Musculoskeletal Research

Improving Mitochondrial Function via CypD Deletion is Effective in Stimulating Bone Formation

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Bone marrow mesenchymal stem cells (BMSCs) are progenitors that differentiate into osteoblasts, adipocytes, and chondrocytes. As BMSCs undergo osteogenic differentiation they upregulate use of their mitochondria and oxidative phosphorylation (OxPhos). Our data and the literature indicate that active mitochondria are required for osteogenesis. Our lab is investigating if strategies aimed at improving mitochondrial OxPhos are effective in stimulating bone formation. One such strategy is inhibition of opening of a non-selective mitochondrial pore called the mitochondrial permeability transition pore (MPTP). MPTP opening is the most common mechanism of mitochondrial dysfunction and is positively regulated by cyclophilin D (CypD). Recently, our lab showed that protecting the mitochondria by genetic removal of CypD protected against age-related bone loss in mice. However, the role of CypD during osteogenic differentiation in physiological and pathological settings has not been fully elucidated. To uncover the role of CypD during osteogenic differentiation, we isolated BMSCs from CypD KO mice and control littermates to perform osteoinduction. Throughout osteogenesis the MPTP closes, which is further desensitized by CypD KO. CypD KO BMSCs show improved mitochondrial function and mineralization during osteogenesis. In addition, CypD KO BMSCs produced larger ossicles following an ectopic bone formation assay, indicating increased osteogenic potential. To uncover the role of CypD in a pathological setting, tibial fractures were done on both CypD KO mice and control littermates. Throughout fracture healing blood and bones were collected. Fractured and unfractured tibiae were analyzed via biomechanical testing, histology, IHC and [redacted] observed that CypD KO mice show enhanced fracture healing in regards to bone biomechanical parameters and bone formation when compared to control littermates, which may result from a truncated cartilage phase and faster ossification phase. This work shows that improving mitochondrial function is effective in stimulating bone formation, highlighting the importance of mitochondria for bone formation.

Poster: 34

Robert Maynard, MS

Program Year: 2014

Advisor and Department: Cheryl Ackert-Bicknell, Department of Orthopaedics

Establishing the functional role of cadherin-like and PC-esterase domain containing 1 (*Cped1*) in the osteoblast.

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Cadherin-like and PC-esterase domain containing 1 (*CPED1*) is an uncharacterized gene with no known function. Human genome wide association studies (GWAS) for bone mineral density (BMD) have repeatedly identified a significant locus on Chromosome 7 that contains the gene *CPED1*, but it remains unclear if this gene could be causative. We set out to characterize *Cped1* in mouse to establish that it could plausibly be able to contribute to BMD. In murine calvarial osteoblasts, *Cped1* expression increases throughout osteoblast maturation and correlates with expression of the extracellular matrix (ECM) proteins osteonectin (*Sparc*, R^2=0.99) and type I collagen (*Col1a1*, R^2=0.83). CPED1 protein putatively contains an N-terminal signal peptide for translocation to the cell surface where it may be secreted and interact with the ECM. We confirmed that CPED1 is present in both the cell lysates and the culture media using a CPED1-FLAG construct, confirming that CPED1 is secreted. To test the function of *Cped1* in vitro, we delivered siRNA to differentiating, calvarial-derived pre-osteoblasts. Knockdown of *Cped1* transcript resulted in a significant increase in *Sp7*, a critical transcription factor for osteoblast maturation. *Sparc* (osteonectin) and Ibsp (bone sialoprotein) expression and protein levels were significantly elevated, suggesting that CPED1 may have a role in the regulation of ECM proteins. To test the function of *Cped1 in vivo*, we generated *Cped1* knockout mice using CRISPR-Cas9. Calvarial osteoblasts from these mice had elevated alkaline phosphatase activity indicative of increased osteoblast activity. Dual-energy x-ray absorptiometry data showed that female knockout mice had increased whole-body and lumbar spine bone mineral content compared to littermate controls. In conclusion, these data demonstrate that *Cped1* is important for osteoblast maturation and ECM protein content, and that *Cped1* has a role in the regulation of bone mass. Our data provide a link between human GWAS and a potential contribution of *Cped1*.

Poster: 35

Madison Doolittle, MS

Program Year: 2015

Advisor and Department: Cheryl Ackert-Bicknell, PhD, Department of Orthopaedics

Investigating *Zbtb40* as a Determinant of Osteoblast Function and Commitment

Madison L. Doolittle[1], Robert D. Maynard[1], Gina M. Calabrese[2], Charles R. Farber[2], Cheryl L. Ackert-Bicknell[1]

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Osteoporosis is a polygenic disorder that occurs due to an imbalance between bone formation and resorption rates. Mineralization by the bone-forming cell, the osteoblast, has been shown to be a highly heritable phenotype, indicating a significant level of genetic regulation over its function. Focusing our genetic studies on bone formation, we previously identified a genomic locus on Chromosome 4 associated with mouse osteoblast mineralization, which lies upstream of the uncharacterized gene Zinc Finger and BTB Domain-Containing 40 (*Zbtb40*). A locus for bone mineral density (BMD) at the homologous region in humans has been identified in multiple previous genome wide association studies. This gene has no known function; however, proteins from the same family are transcription factors that regulate cell differentiation and maturational commitment. Our studies indicate that *Zbtb40* has its highest expression in the initial days of *in vitro* differentiation of both MC3T3-E1 preosteoblasts and primary calvarial osteoblasts, with expression tapering as these cells mature. Knockdown of *Zbtb40* in differentiating MC3T3-E1 cells drastically decreased alkaline phosphatase activity (a marker of osteoblast differentiation) and inhibited mineralization by mature osteoblast-like cells. Furthermore, silencing of *Zbtb40* decreased expression of early osteoblast markers *Col1a1, Runx2*, and *Sp7*. *Zbtb40* knockdown also inhibited expression of *Msx2*, a transcription factor that stimulates commitment of mesenchymal progenitors to the osteoblast over adipocyte lineage. Moreover, we found that expression of *Pparg*, an adipocyte marker, was markedly increased in these cells, suggesting *Zbtb40* loss-of-function down-regulates osteoblast commitment and up-regulates adipogenesis. These data strongly support the hypothesis that *Zbtb40* is the gene underlying these mouse and human genetic loci and that this gene may represent a novel regulator of bone formation, possibly through regulation of mesenchymal cell fate commitment down the osteoblast lineage.

Poster: 36

John Bachman

Program Year: 2016

Advisor and Department: Joe Chakkalakal, PhD; Dept. of Pharmacology and Physiology

Prepubertal skeletal muscle growth requires Pax7-expressing satellite cell-derived myonuclear contribution

Authors: John F Bachman[1,2,3], Nicole D Paris[2,3], Alanna Klose[3], Wenxuan Liu[3,4,], Melissa Schmalz[2,3], Joe V Chakkalakal[2,3,5]

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Pax7- expressing satellite cells (SCs) are a well-defined population of adult muscle stem cells. Quiescent in adult skeletal muscle, SCs can respond to myofiber injury and regenerate the damaged myofiber. However, the contribution of SCs to postnatal skeletal muscle development remains obscure and is deemed largely non-essential post-weaning. Therefore, a role for SCs during prepubertal growth, a period of extensive skeletal muscle development after weaning and prior to the onset of puberty, has not been examined. We hypothesize that SCs not only contribute to prepubertal skeletal muscle development but are essential for the necessary maturation.

Here, we identify a strong correlation between SC-derived myonuclear number and multinucleated skeletal muscle fiber growth during prepuberty. Remarkably, genome-wide RNAseq analysis establishes that post-weaning juvenile and early adolescent (demarked by puberty onset) skeletal muscle have markedly different gene expression signatures. Within just a two-week span, over 900 differentially expressed genes were identified, many of them being related to changes in the extracellular matrix, calcium handling, or metabolism. These distinctions are consistent with extensive skeletal muscle maturation during this essential, albeit brief, phase of pediatric skeletal muscle development. Indelible labeling of SCs with Pax7CreERT2/+; Rosa26nTnG/+ (P7nTnG) mice demonstrated extensive SC-derived myonuclear contribution during prepuberty, with substantial reduction at puberty onset. Prepubertal depletion of SCs in Pax7CreERT2/+; Rosa26DTA/+ (P7DTA) mice reduced myofiber size and myonuclear number, as well as causing deficits in force generation in both fast and slow-contracting muscles.

Collectively, these data demonstrate, for the first time, the critical importance of SCs during prepubertal and early adolescent skeletal muscle growth.

Poster: 37

Katherine Best, MS

Program Year: 2015

Advisor and Department: Alayna Loiselle, PhD, Orthopaedics

Knockout of *NFKB1* Results in Healing Tendon with Increased Scar Tissue Deposition and Decreased Strength

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Healing of acute tendon injuries is characterized by excessive scar tissue deposition and limited tendon regeneration, resulting in healed tendons that are biomechanically weaker and functionally impaired compared to uninjured counterparts. Inflammation is necessary for tendon healing and is postulated to be a key driver of the scar-forming phenotype. Knowledge of inflammatory pathways activated during tendon healing is minimal, restricting development of biological therapeutics. Canonical NF-κB signaling is a pro-inflammatory pathway that has previously been implicated in scar-mediated tendon healing. Examining the contributions of key canonical NF-κB proteins during tendon healing is imperative for understanding the role of NF-κB tendon scar formation. The *NFKB1* protein (p105/p50) constitutes one half of the canonical NF-κB dimer. To test the hypothesis that canonical NF-κB signaling, and specifically *NFKB1*, contributes to scar-mediated tendon healing, 10-12-week-old mice received a flexor digitorum longus (FDL) tendon transection and repair. We have shown that canonical NF-κB signaling is upregulated during tendon healing within 60 minutes of repair in C57Bl/6J mice, implicating NF-κB in tendon healing. Using *NFKB1* knockout (KO), heterozygote (Het), and wildtype (WT) mice, we found that KO mice had a significant decrease in max load at failure compared to Hets and a trending, non-significant decrease compared to WT at D14 ($p=0.0352$ and 0.1030, respectively, n=5-8), indicating that KO tendons were significantly weaker than Het and WT following injury. Histological visualization of collagen at day 14 suggests that KOs exhibit increased collagen deposition at the injury site compared to WT, indicative of an over-abundant scar phenotype. Increased presence of F4/80+ macrophages in the forming scar tissue at day 14 was detected in KO mice compared to WT, implicating macrophages as a possible mechanism for this elevated scar-forming response. With this work, we have shown that *NFKB1* is involved in scar-mediated tendon healing through macrophage-mediated scar tissue deposition.

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Sarah Catheline, MS

Program Year: 2013

Advisor and Department: Jennifer Jonason, PhD, Center for Musculoskeletal Research

Chondrocyte-Specific RUNX2 Overexpression Accelerates Cartilage Degeneration Following Traumatic Injury

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RUNX2 is a transcription factor responsible for regulating the process of chondrocyte hypertrophy and terminal maturation. RUNX2's function is critical during the developmental process of endochondral bone formation, as *Runx2*-deficient mice completely lack mineralized bone, and show either extremely delayed or completely absent chondrocyte hypertrophy. Osteoarthritis (OA) is a debilitating joint disease causing irreversible loss of articular cartilage within synovial joints. Despite the high prevalence of OA, molecular mechanisms underlying onset are unclear. Importantly, enhanced RUNX2 expression is seen in early stages of human OA and in murine models of injury-induced OA. Our preliminary data surprisingly show, however, that postnatal chondrocyte-specific RUNX2 overexpression alone is insufficient to induce OA. However, based on the increase in RUNX2 in articular cartilage during traumatic injury, we hypothesize that chondrocyte-specific RUNX2 overexpression could *predisposes* the knee joint to accelerated degeneration following meniscal ligamentous injury (MLI). At 2 months of age, male *Acan-Cre*$^{+/-}$*ER*^{T2}*; ROSA-Runx2*$^{f/+}$ (RUNX2 GOF) and littermate Cre-negative control mice were given tamoxifen (100µg/g body weight) daily for 5 days (N = 6 per genotype). At 10 weeks of age, mice were subjected to MLI in the right hindlimb, and a sham injury in the contralateral hindlimb. Histology reveals that RUNX2 GOF mice have enhanced cartilage damage and Safranin-O staining loss compared to controls; these findings were confirmed by significantly increased modified OARSI scores and significantly decreased total tibial cartilage areas in RUNX2 GOF mice 2 months post-MLI. RUNX2 GOF mice also show significantly increased TUNEL-positive cells and MMP13 expression 1 month post-MLI in the articular cartilage. We hypothesize that RUNX2 activity is influenced by the oxidative and inflammatory environment following traumatic injury, which could explain why RUNX2 GOF accelerates OA development following injury, but has no effect at homeostasis. These results highlight the contribution of genetic variability to the development of OA and suggest that genetic alterations affecting RUNX2 expression levels may in part predetermine the rate of OA onset following injury.

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David Villani

Program Year: 2017

Advisor and Department: Michael J. Zuscik, PhD, Department of Orthopedics

Prebiotic alteration of the gut microbiome rescues impaired fracture healing in obesity

David A. Villani, Christopher W. Farnsworth, Ashlee MacDonald, Eric M. Schott, Jun Zhang, Alex Grier, Cheryl Ackert-Bicknell, Steven Gill, Hani Awad, John P. Ketz, Robert A. Mooney, Michael J. Zuscik, University of Rochester Medical Center

Purpose: Obesity is a risk factor for delayed fracture healing and fibrous non-union. We have published that high fat (HF) diet-induced obesity in mice leads to delayed tibial fracture repair, possibly through an obesity-induced systemic proinflammatory state that impacts the differentiation of osteoprogenitors during healing. It has been established that the altered gut microbiome in obesity influences systemic inflammation. Since specific prebiotics, including the undigestible fiber oligofructose (OF), can restore a healthy gut microbiome and suppress the systemic inflammation of obesity, we hypothesize that supplementing the HF diet with OF will rescue the impaired fracture healing in obese mice.

Methods: Lean and HF diet-induced obese mice were supplemented with OF or a control fiber (cellulose), and tibia fractures were surgically induced 2 weeks later. Mice were continued on supplements until sacrifice at 21 days post-fracture, with fecal samples collected pre- and post-OF for analysis of microbial abundance by 16S rDNA sequencing. Micro-CT imaging and histomorphometry analysis of stained tissue sections supported study of callus architecture.

Results: Obese mice had larger fracture calluses based on histomorphometry and microCT. This tissue phenotype included a marked increase in callus adiposity, with significant increases in % adipocyte area and adipocyte number. In mice provided OF, callus architecture, size and adiposity were normalized to those of lean mice with reduction in % adipocyte area, and adipocyte number. The rescue by OF corresponded with a profound shift in the gut microbiome. *Bifidobacterium pseudolongum*, a species associated with decreased inflammation, was lost in obesity but rescued by OF. Conversely, proinflammatory species that were increased in obesity, including *Lactobacillus helveticus* and *Staphylococcus sciuri*, were reduced by OF.

Conclusion: We demonstrate for the first time that manipulation of the gut microbiome impacts fracture healing. Data suggest that impaired fracture repair in obesity is linked to an inflammatory process driven by an altered gut microbiome that can be addressed by restoring a healthy microbial profile using dietary prebiotics. Prebiotics may be a simple candidate treatment for a clinical problem that is without a globally accepted therapeutic strategy short of aggressive endocrine and surgical interventions.

Poster: 40

Richard Bell, MS

Program Year: 2014

Advisor and Department: Edward Schwarz, PhD, Orthopaedics

Genetic Ablation of iNOS in TNF-Tg Mice with Inflammatory-Erosive Arthritis Prevents Lymph Node Expansion and Decreases Synovial Infiltrates

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Recent studies in the TNF-Tg mouse model of rheumatoid arthritis (RA) demonstrated a critical role of lymphatic vessel (LV) function in joint homeostasis and arthritis progression, as loss of LV contractions promotes synovitis and erosions due to decrease drainage of joint inflammation. The joint draining lymph nodes (LN) were also found to be a biomarker of arthritic progression, as they expand in volume during the onset of arthritis. It is also known that macrophages and lymphatic endothelial cells express inducible nitric oxide synthase (iNOS), which disrupts the LV contractions that transport immune cells to the draining LNs. Lastly, pharmacological inhibition of iNOS transiently recovers LV contractions in TNF-Tg mice, and maintains flow in the LVs and LNs, which correlates with reduced synovitis and bone damage in inflamed joints. Therefore, we hypothesized that global genetic ablation of iNOS prevents expansion of draining LN, maintains LV contractions, and ameliorates synovitis in TNF-Tg mice. To test this, male and female $iNOS^{-/-}$ xTNF-Tg mice and control littermates ($iNOS^{-/-}$, TNF-Tg, and WT; n=4-12 per group; all data reported as mean ± standard deviation) were examine for LN volume, LV contraction frequency and synovitis. Since stark sexual dimorphism exists in TNF-Tg mice, female LN volume and LV contraction frequency were measured at 2, 3 and 4 months of age, and then knees were collected for histology; while the male mice were assessed at 3, 4, 5 and 6 months, with their knees subsequently collected. No differences were seen in LN volume at the earliest time points in both sexes between TNF-Tg and $iNOS^{-/-}$xTNF-Tg (Female 2mo: 1.6±0.4 vs 1.4±0.5 mm^3; Male 3mo: 2.5±0.6 vs 1.2±0.6 mm^3), however these were all significantly increased from their sex-aged matched WT and $iNOS^{-/-}$ counterparts (WT and iNOS pooled data, Female 2mo: 0.6±0.2 mm^3, $p<0.05$; Male 3mo: 0.6±0.2 mm^3, $p<0.05$). Importantly, both female and male $iNOS^{-/-}$xTNF-Tg LNs were significantly smaller at 4 and 5 months of age compared to their TNF-Tg littermates, respectively (Female 4mo:1.9±0.9 vs 6.3±1.9, $p<0.05$; Male 5mo: 1.6±0.8 vs 5.3±2.4 mm^3, $p<0.05$). Contraction frequency of the LV was also increased at these timepoints for $iNOS^{-/-}$xTNF-Tg compared to TNF-Tg (Female 4mo: 3.2±1.4 vs 2.5±1 contractions/min, $p<0.05$; Male 5mo: 0.6±0.7 vs 0.1±0.1 contractions/min, $p<0.05$). Lastly, female $iNOS^{-/-}$ xTNF-Tg showed significantly less cells within their synovium compared to TNF-Tg counterparts ($1.3x10^4$±$0.5x10^4$ vs $2.6x10^4$±$0.5x10^4$). Male histology is forthcoming. These data indicate an iNOS independent phase of LN expansion precedes an iNOS dependent phase, and that pharmacological inhibition of iNOS may ameliorate RA progression by preventing the full expansion of the LN while increasing LV function, which would increase inflammatory cells egress from the synovium.

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Xi Lin, MS

Program Year: 2015

Advisor and Department: Lianping Xing, PhD, Department of Pathology and Laboratory Medicine

Modulating protein ubiquitination in synovial macrophages is associated with the pathogenesis of osteoarthritis

X. Lin[1,2], H. Zhang[1,2], B.F. Boyce[1,2], L. Xing[1,2]

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Osteoarthritis (OA) is a whole joint disease, including synovial chronic inflammation. We found that mice carrying post-traumatic OA (PTOA) have increased inflammatory M1 macrophages (M1) in the joint, which is attenuated by proteasome inhibition. We hypothesize that distinct protein ubiquitination profile may be associated with macrophage polarization in PTOA. To test this hypothesis, we performed proteomics on purified ubiquitinated proteins isolated from primary M0 (=quiescent) M1 (=inflammatory), and M2 (=anti-inflammatory) macrophages. Thirty-two and 14 proteins were differentially ubiquitinated in M1s and M2s, respectively. Relative to M0s, 317 proteins in M1s and 326 proteins in M2s had 0.5-fold or more ubiquitination. Pathway analyses showed that 16 of the top 20 highly dysregulated pathways were distinctively M1 or M2. To determine if modifying protein ubiquitination affects macrophage polarization, thereby OA pathogenesis, we generated mice deficient ubiquitin E3 ligase Itch and demonstrated that Itch-/- M1s expressed higher pro-inflammatory genes than M0s (*IL-1*: ~35, *iNOS*: ~12 fold). Itch-/- mice developed more severe PTOA, associating with increased F4/80+macrophage infiltration in the synovium (5.86±1.39% vs. 3.39±0.60% in sham). To determine if expression of Itch in macrophages is required for PTOA phenotype in Itch-/- mice, we generated macrophage conditional Itch knockout mice (cKO). Similar as Itch global knockout mice, cKO mice had more severe PTOA with a significant increased OARSI score (cKO: 4.37±0.96 vs. 3.20±0.48 in sham). More M1s were detected in PTOA synovium of cKO mice (cKO: 34.71±6.37% vs. 19.90±1.88% in sham). In conclusion, macrophage polarization is associated with differential protein ubiquitination, and depletion of Itch in macrophages exacerbates PTOA progression by promoting M1 polarization. Alternation of protein ubiquitination and degradation may contribute to PTOA pathogenesis by affecting macrophage polarization in synovium, which may represent a new therapeutic targeting pathway for OA.

Poster: 42

Jinbo Li, MS

Program Year: 2015

Advisor and Department: Brendan F. Boyce M.D., Department of Pathology and Laboratory Medicine

RANKL+ plasmacytic B and TGFβ+ myeloid cells are attracted to bone marrow during aging by a TRAF3-dependent mechanism to increase bone resorption, decrease bone formation and promote osteoporosis

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Department of Pathology and Laboratory Medicine, URMC

Levels of TGFβ1 and RANKL increase in bone marrow (BM) with age and induce lysosomal degradation of TNF receptor-associated factor 3 (TRAF3), a negative regulator of NF-κB signaling, in osteoblast (OB) and osteoclast (OC) precursors, respectively. Mice we generated with TRAF3 conditionally deleted in mesenchymal lineage cells (using Prx-1 Cre) or myeloid cells (using Lys-M Cre) develop accelerated osteoporosis with aging. Low-grade chronic inflammation (LLCI) also induces bone loss during aging; we hypothesize that TGFβ1 and RANKL mediate this loss. We found that BM cells (BMCs) comprise 62±5% of TGFβ1 and 75±7% of RANKL expression in leg bones from 22-m-old mice with significantly higher levels of TGFβ1 (16±3 vs 10±1 ng/ml) and RANKL (644±50 vs 475±19 pg/ml) than in BM from 4-m-old mice. 78% of TGFβ1+ BMCs were CD11b+Ly6G^{hi}Ly6C+CCR5+ myeloid cells and 70% of RANKL+ BMCs were B220^{hi}IgM+IgD+CD138+CXCR4+ plasmacytic B cells. The % and absolute # of these cell populations were 2-fold higher in BM from old than young mice. In addition, protein levels of CCL5, a CCR5 ligand, and CXCL12, a CXCR4 ligand, were increased 12- and 16-fold, resp., in BMCs from old than young mice. CCL5 and CXCL12 mRNA levels were 4- and 7-fold higher, resp., in CD45- mesenchymal cells from BM from old mice, but not in CD45+ hematopoietic cells. Of note, mice with TRAF3 conditionally deleted in mesenchymal lineage cells, but not in myeloid cells, had elevated CCL5 and CXCL12 expression, increased BM RANKL+ plasmacytic B and TGFβ1+ myeloid cells, enhanced OC formation and reduced OB differentiation, similar to changes detected in old WT mice. Consistent with these findings, old WT mice treated with the FDA-approved drugs, Plerixafor (P), a CXCR4 antagonist, or Maraviroc (M), a CCR5 antagonist, had significantly reduced absolute #s of plasmacytic B cells (by 59% for P) and myeloid cells (by 49% for P; 57% for M) in BM, and increased vertebral trabecular bone mass (BV/TV: 17±4% for P, 15±4% for M vs 12±2% in Ctrl). Our findings suggest that RANKL+ plasmacytic B cells and TGFβ1+ myeloid cells are attracted to BM in response to increased CXCL12 and CCL5 expressed by mesenchymal cells deficient in TRAF3 either genetically or during aging to promote LLCI-mediated bone loss. Plerixafor and Maraviroc are potential new therapies for osteoporosis that could inhibit bone resorption and enhance bone formation by keeping these RANKL- and TGFβ-expressing cells in blood and away from BM.

Poster: 43

Allison Li, MS

Program Year: 2014

Advisor and Department: Laura Calvi, MD, Medicine M&D Endocrinology-Metabolism Div.

Myelodysplastic syndrome (MDS) induces remodeling of mesenchymal-osteolineage cells in the bone marrow microenvironment and impairs normal hematopoiesis

Allison J. Li[1,2,4], Benjamin J. Frisch[1,4], Rhonda Staversky[1,4], Mark W. LaMere[1,4], Kathleen E. McGrath[3], Archibald S. Perkins[2,4], Michael W. Becker[1,4], Jane L. Liesveld[1,4], James Palis[3], Laura M. Calvi[1,4]

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Myelodysplastic syndromes (MDS) are malignant disorders of hematopoietic stem cells (HSC) hallmarked by marrow failure due to defective hematopoiesis. Despite being the most common myeloid neoplasm in the U.S., MDS remains a therapeutic challenge due to a paucity of efficacious agents and difficulties of targeting the heterogeneous genetic defects in malignant hematopoietic cells. As a result, MDS leads to significant morbidity and mortality due to multi-lineage blood cytopenias and transformation to acute leukemia.

Work by our lab and others indicate that abnormalities in the bone marrow microenvironment (BMME) may be common disease features contributing to MDS initiation and progression. Under normal physiologic conditions, mesenchymal-osteolineage cells in the BMME are critical regulators of HSC function, suggesting that they may be novel therapeutic targets to enhance hematopoietic function. While in vitro studies of human MDS suggest that BMME cells are abnormal, the MDS BMME has not been assessed in a robust in vivo model, limiting our understanding of bi-directional interactions between MDS and its BMME during disease pathogenesis.

We previously reported that the BMME in the NUP98-HOXD13 transgenic murine model of MDS exhibited expansion of non-functional osteoblastic-lineage cells (OBC) and precursor multipotent stromal cells (MSC). BMME changes in MDS mice are associated with hematopoietic dysfunction characterized by HSC loss, dysmyelopoiesis, and blood cytopenias. To determine if MDS can initiate BMME alterations, we transplanted MDS or wild-type (WT) donor marrow into irradiated WT recipients, introducing MDS marrow into the initially normal BMME of WT recipients. Post-transplant, OBCs and MSCs were increased in MDS transplanted mice, indicating that interaction with MDS induced mesenchymal-osteolineage cells to take on features of an MDS BMME. Concurrent with BMME remodeling, HSCs were depleted in MDS transplanted compared to WT transplanted mice. HSC depletion was also observed when WT recipients were transplanted with MDS marrow mixed with WT GFP+ marrow to result in only 4% contribution by MDS cells to reconstituted recipient marrow. Thus, even when recipient marrow is comprised of over 90% normal hematopoietic cells, the presence of MDS cells results in HSC depletion. These data suggest that MDS-initiated remodeling of the mesenchymal-osteolineage BMME impairs normal hematopoiesis, identifying the BMME as a potential therapeutic target to improve hematopoietic function in MDS.

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Jerry Saunders II, MS

Program Year: 2015

Advisor and Department: Dr. James Palis, MD, Department of Pediatrics

16, 16-Dimethyl prostaglandin E_2 ($dmPGE_2$) and lisinopril cooperatively improve survival and mitigate injury in hematopoietic-acute radiation syndrome (H-ARS)

Jerry Saunders II[1,2], Anne D. Koniski[3], Seana C. Catherman[3], Katherine H. Fegan[3], Paul D. Kingsley[3], Kathleen E. McGrath[3], James Palis[3]

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H-ARS presents as leukopenia and thrombocytopenia following exposure to ionizing radiation. Thrombocytopenia increases the risk of mortality from excessive hemorrhage. **Our long-term goal is to develop medical countermeasures to improve survival and mitigate thrombocytopenia in H-ARS, particularly in the context of nuclear terrorism or radiological accidents.** $dmPGE_2$ improves survival and platelet recovery after total body irradiation (TBI). Various angiotensin-converting enzyme (ACE) inhibitors mitigate delayed effects of TBI to lung and kidney vasculature. Preliminary studies in our laboratory demonstrated that combined treatment with $dmPGE_2$ (given IP at 24 hours post-TBI) and the ACE inhibitor lisinopril (orally beginning at day 7 post-TBI) improves survival in lethally irradiated mice (7.75 Gy, LD 90/30) greater than either agent alone. Using a sublethal TBI model, we tested the hypothesis that combined treatment with $dmPGE_2$ and lisinopril enhances recovery of the megakaryocyte (MK) lineage greater than either agent alone. Platelet recovery was enhanced by combined therapy 10 days after 4 Gy TBI. $dmPGE_2$ alone improved recovery of both marrow MKs and circulating platelets. In contrast, lisinopril alone did not affect recovery of the MK lineage. The physical association between marrow MKs and sinusoidal vascular endothelial cells (ECs) is essential for thrombopoiesis. Consistent with radiation exposure injuring ECs in multiple tissues, the number of $Lin^- CD45^- CD31^+ Sca\text{-}1^-$ cells (sinusoidal ECs) was significantly decreased in marrow 10 days after 4 Gy TBI relative to unirradiated controls. Despite the marked reduction of sinusoidal ECs, we found normal numbers of sinusoidal vessels examined by immunohistochemistry. Since ACE inhibitors can mitigate vascular damage after TBI and PGE_2 can regulate endothelium, we tested the hypothesis that lisinopril and $dmPGE_2$ cooperatively promote the recovery of sinusoidal ECs after TBI. Combined treatment with $dmPGE_2$ and lisinopril accelerated recovery of $Lin^- CD45^- CD31^+ Sca\text{-}1^-$ (sinusoidal) EC numbers to normal levels by 10 days after 4 Gy TBI. Taken together, these data support the concept that $dmPGE_2$ and lisinopril may improve survival in H-ARS by promoting the recovery of the MK lineage and of sinusoidal endothelium.

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Robert Hoff, MS

Program Year: 2012

Advisor and Department: Dirk Bohmann, PhD, Department Biomedical Genetics

Measuring Stochastic Contributions to Age Related Nrf2 Pathway Dysfunction Using Quantitative Single-Cell Reporters

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

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

Aging is a multi-factorial process that results in the progressive dysfunction of organismal fitness. Aging is considered a result of both environmental and genetic influences. However, isogenic model organisms raised in identical environments have significant variability in individual lifespan. Therefore, stochastic processes such as biomolecule damage and epigenetic changes that occur randomly must play a role in aging. The present study attempts to address whether stochastic processes impact age related Nrf2 pathway dysfunction. Nrf2 is a transcription factor that regulates critical antioxidant genes both basally and in response to stress. It has been shown in several model organisms that Nrf2 is critical for healthy aging and that increased Nrf2 function can prolong lifespan. Nrf2 becomes dysfunctional with age and fails to upregulate target genes in response to stress. We have found that loss of Nrf2 inducibility correlates with changes in epigenetic state at target gene loci. However, it not known if these changes occur because of a stochastic process at individual loci or are due to genome wide changes in chromatin due to aging. To test these possibilities, we have generated transgenic Drosophila expressing two different reporters (nuclear localized GFP and dsRed) under the control of Nrf2 regulated AREs (Antioxidant Response Element) using site-specific integration. We predict that both reporters should be equally responsive in cells of young tissue. However, a stochastic model of loss of inducibility would predict that the reporters should behave differentially in old tissue in response to an inducer. However, in the context of more general cellular dysfunction, both reporters should be equally unresponsive. Current data shows a coordinated loss of inducibility, suggesting that loss of Nrf2 target gene induction is possibly downstream of more broad changes in epigenetic state.

Poster: 46

Sara Blick

Program Year: 2017

Advisor and Department: Michael Elliott, PhD, Microbiology and Immunology

Regulation of Phagocytic Exhaustion in Macrophages

Sara Blick[1], Jonathan Pinney[2], Charles Chu[2], Michael R. Elliott[2], Clive Zent[2]

[1]Department of Pathology, University of Rochester, School of Medicine & Dentistry, [2]Department of Microbiology and Immunology

Macrophages are innate myeloid cells that play a critical role in infection clearance and immunity. Macrophages are well known for executing their function as professional phagocytes through numerous different mechanisms including efferocytosis, Fc-mediated and complement-mediated phagocytosis. This project focuses on Fc-mediated phagocytosis, which has been used as a clinical application in cancer immunotherapy. Monoclonal antibodies (mAb) have been developed to promote the phagocytic Fc-mediated mechanism, thus allowing macrophages to efficiently engulf tumor cells through antibody-dependent cell-mediated phagocytosis (ADCP). It has been established, through ADCP, that macrophages undergo a cycle of engorgement until they become saturated with cellular contents. After saturation, macrophages digest and break down the engulfed contents, which is known as the attenuation stage. After the attenuation stage, macrophages undergo a stage of recovery and no longer phagocytose – a novel phase of phagocytic exhaustion we have termed hypo-phagocytosis. This hypo-phagocytic stage is still not well understood. By better understanding hypo-phagocytosis, we can modulate phagocytosis and ultimately prevent hypo-phagocytosis, and allow for improvements in monoclonal antibody therapeutics. The goal of this project was to investigate if the macrophage's phagocytic rate could be modulated to avoid the hypo-phagocytic stage by using lower doses of mAb. Bone marrow derived macrophages (BMDM) were isolated from C57BL/6 mice and cultured for one week, re-plated, and labeled with TAMRA for visualization. Thymocytes, used as target cells, were opsonized with anti-CD-90.2 antibody at varying concentrations. Live cell imaging was carried out to measure void formation (phagocytosis) in the macrophages using a customized software analysis approach. Macrophage colony stimulating factor (M-CSF) was added to ensure viability of macrophages and to see if the phagocytic amount would increase with M-CSF stimulation. The use of anti-CD-90.2 mAb in increasing concentrations showed a dose-dependent increase in phagocytosis. The addition of M-CSF increased the amount of void formation compared to macrophages without M-CSF stimulation. Ultimately, regardless of the lower dose of mAb and the addition of M-CSF, macrophages still reached the hypo-phagocytic stage. The data provide insight into the molecular mechanisms that govern phagocytic exhaustion.

Poster: 47

Melissa Glasner, MS

Program Year: 2014

Advisor and Department: Catherine Ovitt, PhD, Biomedical Genetics and Center for Oral Biology

Identification of Ascl3 as a Potential Marker of Solitary Chemosensory Cells in the Salivary Gland

Melissa Glasner[1,3], Pei-Lun Weng[1,3], Ashwini Manjunatha[3], Jill Kraemer[4], Catherine Ovitt[1,2,3]

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Immune regulation in epithelial tissues such as the lung and small intestine is mediated by single neuroendocrine cells called solitary chemosensory cells (SCCs). SCCs have been demonstrated to act as sentinel cells, and react to external environmental stimuli, leading to activation of immune responses. Common markers of SCCs are members of the canonical taste transduction pathway and include, transient receptor potential cation channel subfamily M member 5 (TRPM5), type 3 inositol 1,4,5-trisphosphate receptor (IP3R3), phospholipase C Beta-2 (PLCB2), and G protein subunit alpha transducin 3 (GNAT3).

Evidence from our laboratory suggests that SCCs exist in the salivary gland and are marked by the salivary gland specific transcription factor, Ascl3. In the olfactory epithelium, we previously demonstrated, using the Ascl3EGFP-Cre/+ R26TdTomato +/- reporter strain, that Ascl3 cells co-express markers of chemosensory cells including TRPM5, PLCB2, and IP3R3. In the salivary glands, we also find that Ascl3 cells in the ducts co-express markers of chemosensory cells, including IP3R3 and PLCB2. Furthermore, specific ablation of Ascl3 expressing cells using the Ascl3EGFP-Cre/+ R26DTA+/- model results in a loss of IP3R3 and PLCB2 in the submandibular gland duct. In addition, following stimulation with the bacterial endotoxin Lipopolysaccharide, we observe a decrease in neutrophil and macrophage recruitment and a decrease in mast cell activation in Ascl3 cell-ablated glands, relative to WT.

Taken together, these results show that Ascl3 cells co-express markers of SCCs and may be important for initiating an innate immune response. Further characterization of these cells will yield better understanding of the salivary gland immune system regulation, and in addition, shed light on mechanisms of salivary gland pathologies.

Poster: 48

Cynthia Tang

Program Year: 2015

Advisor and Department: Dr. Ruchira Singh, PhD, Department of Ophthalmology

Delineating the role of CLN3 at the Photoreceptor-RPE interface in the retina

Cynthia Tang[1,2], Sonal Dalvi[1], Chad Galloway[1], Celia Soto[1], Ruchira Singh[1,3,4]

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Mutations in *CLN3* lead to Juvenile neuronal ceroid lipofuscinosis (JNCL, Batten Disease), a disease characterized by vision loss in early childhood followed by neurodegeneration. Histology of post-mortem eyes from JNCL patients reveals substantial retinal degeneration, but the cell type and mechanism driving retinal degeneration are unknown. Similarly, there is conflicting data from animal models of JNCL, including *Cln3*$^{-/-}$ mice, which suggest both retinal ganglion cells and photoreceptor dysfunction in the disease. Additionally, although it is known that CLN3 is expressed ubiquitously in the retina, the precise function and role of CLN3 has yet to be determined. To understand the role of CLN3 in retinal homeostasis and JNCL pathophysiology, we have generated human induced pluripotent stem cell (hiPSC)-derived retinal cells, both neural retina and retinal pigment epithelium (RPE), from patients and control (unaffected) individuals. Our preliminary studies of control and JNCL patient-derived hiPSC-RPE, reveal that JNCL patient-derived hiPSC-RPE has molecular and structural alterations in apical microvilli. The RPE provides essential support for photoreceptors by carrying out functions such as phagocytosis of shed photoreceptor outer segments and recycling of retinoids for the visual cycle. Since efficient exchange of molecules between photoreceptors and RPE depends on RPE apical microvilli, we hypothesize that CLN3 is expressed in the microvilli of human RPE and is central to the RPE-photoreceptor interaction. To test this hypothesis, we performed CLN3 co-localization studies and assessed the impact of apical microvilli defects on biomolecule uptake in JNCL patient hiPSC-RPE. Our findings reveal that CLN3 is expressed in RPE microvilli, and furthermore, JNCL patient hiPSC-RPE has defects in the phagocytosis of photoreceptor outer segments. Together, our results suggest a possible role for CLN3 in the RPE and for the RPE in JNCL pathogenesis, and thus, has implications for the development of rational therapies for JNCL.

Poster: 49

Olivia Marola

Program Year: 2016

Advisor and Department: Richard T. Libby, PhD, Department of Ophthalmology

Endothelin signaling in glaucomatous neurodegeneration

Olivia J. Marola[1,2], Stephanie B. Syc-Mazurek[2], Gareth R. Howell[3], Richard T. Libby[2]

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Blindness in glaucoma is caused by the loss of retinal ganglion cells (RGCs), the output neurons of the retina. The cellular and molecular events that cause RGC death in glaucoma are incompletely understood. In DBA/2J mice, which develop glaucomatous neurodegeneration as a result of chronic ocular hypertension, endothelin (*Edn*) is upregulated >10 fold in retinas prior to the onset of glaucomatous damage. Antagonizing EDN receptors in DBA/2J mice provided significant protection from glaucomatous neurodegeneration. Despite its promise in glaucoma pathogenesis, the mechanisms of EDN-induced glaucomatous neurodegeneration remain undefined. Thus, elucidating the RGC-intrinsic apoptotic signaling pathways triggered in response to EDN signaling will be an important step in defining the molecular mechanisms of glaucomatous neurodegeneration and can potentially indicate therapeutic targets. We have previously shown that DDIT3 and JUN are major regulators of RGC death after axonal injury—a critical, early insult in glaucoma. Furthermore, we have shown that JUN is important in RGC death in a model of chronic ocular hypertension. Here, we examine whether JUN and/or DDIT3 are important regulators of EDN-induced RGC death. To study the mechanisms of EDN-induced RGC death, C57BL/6J mice were intravitreally injected with EDN1 or vehicle (PBS). EDN1 caused JUN activation (pJUN) in RGCs 3 days following insult, and active caspase 3 (cCASP3) was observed in RGCs from 3-14 days following insult. Four weeks following injection, EDN1 reduced RGC density relative to PBS controls (given %RGC survival relative to respective PBS control± SEM: 80% ±5%; p=0.0001). Interestingly, EDN1 induced JUN activation in wedge-shaped patterns radiating from the optic nerve head, mirroring patterns of RGC loss observed in glaucoma patients and in mouse models of glaucoma. In mice with *Jun* deficient retinas, EDN1 caused less RGC death compared to WT injected animals (89%± 6%; p=0.220). In contrast, *Ddit3* deficiency conferred no protection from EDN1 insult (81% ±6%; p=0.017). Together, these data suggest that JUN, but not DDIT3, is an important mediator of EDN-induced RGC death. Furthermore, these findings are in contrast to the intrinsic mechanisms of RGC death important in other models which show a role for DDIT3 in glaucoma. This suggests that perhaps ocular hypertension causes several different glaucoma-relevant injuries, which are mediated by independent RGC-intrinsic mechanisms.

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Felicia A. Gilels

Program Year: 2016

Advisor and Department: Amy E. Kiernan, PhD, Department of Ophthalmology

The role of Notch receptors in maintenance and function of cochlear sensory cells.

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The mammalian inner ear is a complex sensory organ that detects auditory and balance information through six sensory organs. Inner ear sensory regions are comprised of two major cell types, sensory hair cells and their associated supporting cells. Notch signaling is well established at determining the proper formation of hair cells and supporting cells, through a process known as lateral inhibition, where signaling from the default cell type (hair cell) prevents its neighbors from adopting the same cell fate. Interestingly, the Notch ligand, Jagged1 (JAG1) is expressed in supporting cells, which is inconsistent with the lateral inhibition model. We hypothesize instead that JAG1 may play a role in the differentiation, maintenance or function of cochlear supporting cells. Here, we show that conditional deletion of *Jag1* (*Jag1-cko*) in supporting cells postnatally results in hearing loss. To further investigate the molecular basis of *Jag1-cko* induced hearing loss, RNA-seq analysis was performed on control and *Jag1*-deleted cochleae. Pathway analysis of *Jag1-cko* inner ears identified *Diaph3*, a gene involved in auditory neuropathy and hair cell stereocilia integrity, as significantly upregulated. Interestingly, scanning electron microscopy of the sensory regions in *Jag1-cko* revealed similar stereocilia defects as those observed in *Diaph3* gain-of-function mouse models. This indicates a novel role for Jag1/Notch signaling in stereocilia integrity through suppression of *Diaph3*. To interrogate whether *Diaph3* suppression can be mediated directly as a result of Notch signaling, we are conditionally deleting Notch receptors in the hair cells or supporting cells in order to determine which expression parameters phenocopy the Jag1 hearing loss. Preliminary results demonstrate that supporting cell specific *Notch1* deletion causes profound deafness. Current studies focus on whether the *Notch1-deleted* phenotype is similar to the *Jag1-cko* deafness or whether other receptors could be mediating aspects of the signaling pathway.

Poster: 51

Courtney Kellogg

Program Year: 2016

Advisory and Department: Amy Kiernan, PhD, Department of Ophthalmology

Multiple roles of *Jag1* during Sensory Cell Development in the Cochlea

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The sensory regions of the inner ear are composed of hair cells and supporting cells, critical cell types for the function of hearing and balance. It has been well established that the Notch signaling pathway requires cell-to-cell contact, which is critical for mediating the cell fate decision between adopting a hair cell and supporting cell fate, a process called lateral inhibition. However, there is at least one ligand of Notch, Jagged1 (Jag1) that is expressed prior to differentiation and has been implicated in sensory specification. *Jag1* is initially expressed in the prosensory region and becomes localized to the supporting cells during differentiation. This led us to our question: does the Notch ligand Jag1 have multiple roles during inner ear development? To address this question, we deleted *Jag1* at different time points embryonically, to dissect the function of *Jag1* during sensory region development in the cochlea. Interestingly, deletion of *Jag1* at E11.5 leads to a loss of supporting cells (SC) and outer hair cells (OHC) along with a duplication of inner hair cells (IHC). In contrast, deletion of *Jag1* at E14.5 causes a duplication of IHC but normal OHC and SC phenotype. Given the differences in phenotypes depending on when the deletion occurs, we believe the earlier phenotype correlates to a role in sensory specification, whereas the later role likely corresponds to a loss of boundary formation. Currently, we are performing marker analysis to better understand these phenotypes, as well as auditory testing to determine how these patterning defects change the responses of the cochlea. These data will give us a better understanding of the molecular mechanisms involved in pattern formation in the mammalian cochlea.

Poster: 52

David Richardson

Program Year: 2017

Advisor and Department: John J. Foxe PhD, Neuroscience

Mobile Brain/Body Imaging (MoBI) Assessments of Cognitive-Motor Interference in Alzheimer's Disease

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Walking requires continuous integration of proprioceptive, vestibular, and visual information into the planning, initiation, and calibration of motor commands. While walking commonly comprises a symphony of actions that individuals can conduct even with additional preoccupations, there is growing evidence that attentional shifts towards cognitively demanding tasks or sensory stimuli can interfere with execution of physical tasks (and vice versa). This notion of cognitive load dominating allocable cognitive resources is of particular importance to several patient populations, including individuals with cognitive deficits ranging from temporary to permanent, and individuals predisposed to falling. Patients with Alzheimer's Disease (AD) or its precursor, amnestic Mild Cognitive Impairment (aMCI), display both of these characteristics. Our study aims to explore what limits an individual's ability to adapt to a discrete cognitive load, improve upon existing capabilities for assessing fall risk, and discover novel methods for early identification of AD and aMCI. In pursuit of these aims, we will employ Mobile Brain/Body Imaging (MoBI), a technology combining EEG with high resolution motion capture software to relate the brain's electrophysiological events to kinematic data. Previous studies out of the Cognitive Neurophysiology Lab of Dr. John Foxe at the Albert Einstein College of Medicine utilized MoBI to demonstrate age-related differences in cognitive task performance and event-related potential modulations during dual-task loads, suggesting a constrained neurophysiological flexibility in aging populations. Our study will attempt to build upon these findings. Subjects will participate in a series of Go/NoGo tasks in response to mixed auditory and visual stimuli while sitting, standing, or walking on a treadmill. In this manner, a neural 'stress test' will be administered to expose vulnerabilities within the cognitive and motor domains, similarly to how a cardiac stress test might reveal any cardiovascular susceptibilities. Given the ability of MoBI to simultaneously assess perturbations in electrophysiological, cognitive, and physical domains, we will strive to uncover novel biomarkers indicative of aMCI and AD.

Oral Presentation

Nisha Patel, DO

Resident: PGY-4

Microarray CGH-SNP Analysis Detects Frequent Chromosomal Abnormalities Indicating Clonal Cytopenia(s) in Patients With Indeterminate Bone Marrow Dysplasia - An Institutional Study Of 94 Cases

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Background
Disorders of ineffective clonal hematopoiesis can present with indolent clinical presentations, subtle morphologic bone marrow changes, and subdiagnostic cytogenetic abnormalities that can cause diagnostic uncertainty. While conventional karyotype and fluorescence in-situ-hybridization (FISH) are considered standard in evaluating cytogenetic abnormalities, emerging data shows comparative genomic hybridization (CGH) and single nucleotide polymorphism (SNP) array can potentially identify clinically significant genetic lesions. We examined the ability of combined microarray CGH-SNP analysis (aCGH-SNP) to detect DNA copy number variations (CNVs) and regions of homozygosity (ROH) in normal karyotype individuals undergoing evaluation for unexplained cytopenias.

Design
Ninety-four (94) bone marrow samples deemed indeterminate for dysplasia were collected over a 3 year period (2014-2017) from patients referred for unexplained cytopenias. Inclusion criteria included “normal” or non-clonal karyotype abnormalities by standard cytogenetics. aCGH-SNP analysis was performed using a cancer targeted array (4x180K - Agilent Technologies) designed by the Cancer Genomics Consortium. The platform contains approximately 120K oligonucleotides that provide high-density coverage for clinically relevant oncogenes, telomeres and pericentromeric regions, and 60K SNP probes (MAF >5.0), providing resolution of 5-10 Mb for ROH. The cut-off for CNVs was 250kb, containing at least one targeted gene, with confirmation by FISH. Results were compared to 18 normal bone marrow controls.

Results
Thirty-seven (37) patients (39% of total) were positive for a chromosomal abnormality by aCGH-SNP analysis. Twenty cases (21% of total) were positive for CNVs, and 17 cases (18% of total) were positive for ROH alone. CNVs among patients included a 555 kb deletion at 4q24 (TET2 gene); a 6.4 Mb deletion at 12p13.31 (ETV6 gene); a 25.8 Mb loss at 20q11.23; a 91.8 Mb ROH (UPD7q; EZH2 gene) and 135.9 Mb ROH (UPD4q; TET2 gene). All of these regions are implicated in MDS pathogenesis.

Conclusion
More than a third of patient samples were found positive for CGH-SNP findings in the presence of a normal karyotype, including causative genes associated with MDS. Our data supports utilization of microarray CGH and SNP array in evaluating unexplained cytopenias. Furthermore, it suggests an alternative approach to detect clonal cytopenias of uncertain significance, and has implications for early detection of myelodysplastic syndromes.

Oral Presentation

Alexandra Danakas, DO

Resident: PGY-2

Real Time Cytopathology Feedback (RTCF) versus traditional Rapid On-Site Evaluation (ROSE) for Endobronchial Ultrasound Guided Fine-Needle Aspiration (EBUS-FNA) of mediastinal lymph nodes (MLN)

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Background:
Real Time Cytopathology Feedback (RTCF) was established as a cytology service method to enhance the traditional Rapid On-Site Evaluation (ROSE), to pause the EBUS-FNA to incorporate real time cytopathology input, aimed to obtain minimally bloody, non-diluted, well-smeared slides and ample material for the best possible diagnosis and ancillary studies triage. The RTCF is based on the cytopathologist directions to the interventionist based on lesion's ultrasound physical characteristics, and gross and microscopic material quality obtained in a timely fashion. Use of suction, rate of needle movement coupled with time in the lesion, speed of flashing of material on glass slides, and cytologic interpretations are part of the RTCF. A team composed of cytotechnologists, a cytology fellow and two cytopathologist was created. Traditional ROSE is generally restricted to verbalization of the microscopic findings from the cytopathologist to the interventionist. Our aim is to compare the diagnostic accuracy between RTCF and ROSE for EBUS-FNA of mediastinal lymph nodes (MLN).

Design:
An IRB exempt retrospective review of EBUS-FNA cases performed from January 2017 to July 2017. The final diagnoses, number of non-diagnostic samples (NDS), and pertinent patient clinical information were reviewed using our pathology computer software. The number of NDS using RTCF and ROSE was analyzed, compared, and tabulated using chi-square test.

Results:

400 MLN from 190 patients were collected. The number of MLN per patient range from 1 to 8 with an average of 2 MLN per patient.

286 MLN samples from 134 patients had ROSE, resulting in 33 NDS (33/286; 11.5 %) from 24 patients (24/ 134; 18%).

The RTCF group includes 114 MLN samples from 56 patients, resulting in 3 NDS (3/114; 2.6%) from 2 patients (2/56; 3.6%).

A highly significant difference **(p<0.01)** in the NDS was found between ROSE and RTCF when tabulated for MLN (11.5% versus 2.6%) as well as for patients (18% versus 3.6%).

Results Table 1: Comparison between RTCF and ROSE to assist EBUS-FNA mediastinal lymph node sampling

Diagnostic Method	Total number of M	Number of no MLN	Total number patients	Number of pati with non-dx MLN
RTCF	114	3 (2.6 %)	56	2 (3.6 %)
ROSE	286	33 (11.5 %)	134	24 (18 %)

EBUS-FNA = Endobronchial ultrasound guided – fine needle aspiration.

RTCF = Real Time Cytopathology Feedback

ROSE = Rapid On-Site Evaluation

Conclusion:
RTCF significantly improved the diagnostic accuracy of EBUS-FNA for mediastinal lymph node staging when compared with traditional ROSE.

Oral Presentation

Chad A. Hudson, MD, PhD

Fellow: Hematopathology

Clinical utility of classical and non-classical monocyte percentage in the diagnosis of Chronic Myelomonocytic Leukemia

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OBJECTIVE: To determine if a clinically applicable flow cytometry methodology could identify chronic myelomonocytic leukemia (CMML) cases.

METHODS: Monocyte subset screening (CD14/CD16 expression) was performed on 68 blood and 25 bone marrow specimens with a monocytosis and/or flagged as possible CMML. Fifty thousand total events were obtained per case. Cases were categorized as CMML, atypical chronic myeloid leukemia (aCML), or non-CMML+non-aCML by clinicopathological diagnosis.

RESULTS: The methodology could differentiate blood and bone marrow CMML cases from non-CMML+non-aCML but not the 3 aCML cases in the clinical setting. Further, a decreased percentage of non-classical monocytes ($CD14^{dim}CD16^{+}$) showed better sensitivity than the previously described approach that relied on the increased percentage of classical monocytes ($CD14^{bright}CD16^{-}$).

CONCLUSION: Quantification of monocyte subsets is useful in clinical practice as a diagnostic marker of CMML in blood and bone marrow specimens. Furthermore, the percentage of non-classical monocytes should be included in the analysis of monocyte subsets.

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