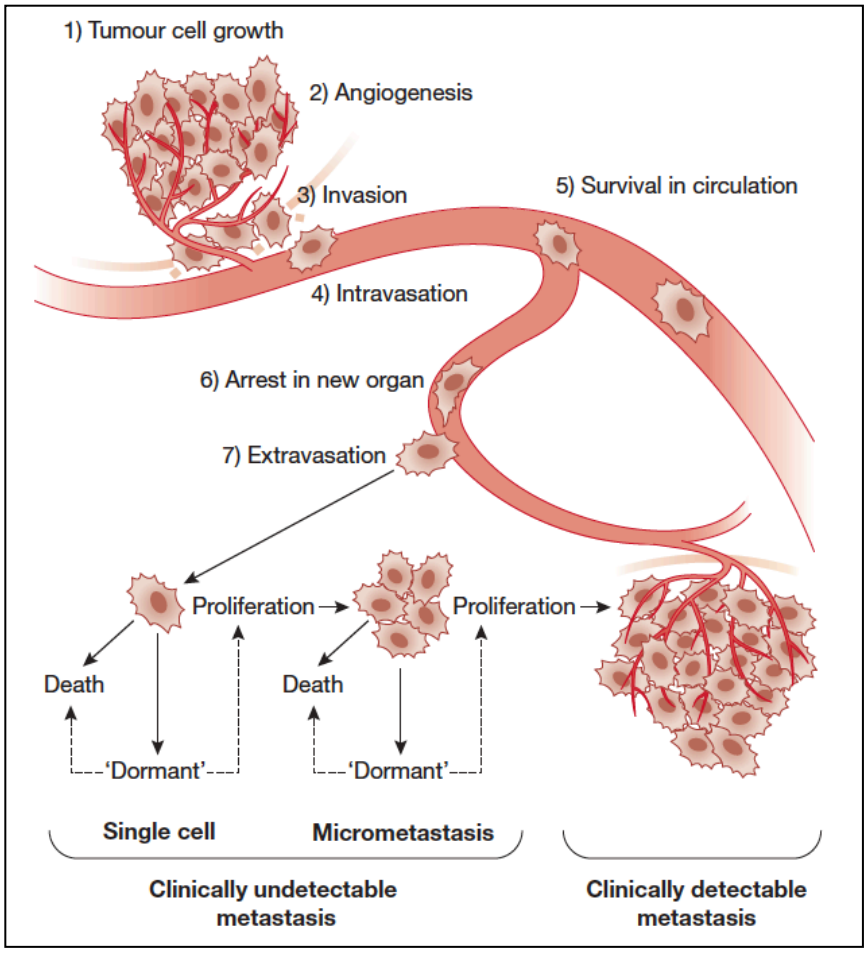
Sixth Annual Cancer Biology Research Retreat

Monday, May 18, 2015
UMB SMC Campus Center
University of Maryland, Baltimore, MD



McGee et al., EMBO Rep. 7:1084 (2006)

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University of Maryland Marlene and Stewart Greenebaum Cancer Center
University of Maryland School of Medicine Graduate Program in Life Sciences
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WELCOME

Welcome to the Sixth Annual Cancer Biology Research Retreat at the University of Maryland. This is an opportunity for graduate students, postdoctoral and clinical research fellows and faculty involved in cancer research to interact and share research ideas and techniques in an informal and relaxed environment. The Organizing Committee would like to especially thank the Retreat Sponsors for their financial support and the Keynote Speaker for his participation in today's Research Retreat.

Enjoy the day,
Michele Vitolo and Jeff Winkles

Cancer Biology Research Retreat Organizing Committee

Kristi Chakrabarti, Chair
Tyler Gable
Greg Conway
Nina Connolly, Ph.D.
Nadire Duru, Ph.D.
Lindsay Hessler, M.D.
Michele Vitolo, Ph.D., Faculty Advisor
Jeff Winkles, Ph.D., Faculty Advisor
Marcina Garner, Molecular Medicine Program Manager

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PROGRAM

8:45 am-9:25 am Check-In and Continental Breakfast, *Second Floor Lobby*

9:25 am Welcome, *Room 208*

Oral Session I: Predoctoral, *Room 208*

Chair: **Greg Conway**

9:30 am **Christina Ross**
The mRNA-destabilizing protein tristetraprolin suppresses tumorigenic phenotypes in a triple negative breast cancer cell model via a non-canonical mechanism (Abstract #23)

9:45 am Discussion

9:50 am **Nidal Muvarak**
Combination of DNA methyltransferase and PARP inhibitors as a novel therapy strategy for poor prognosis acute myeloid leukemia (Abstract #18)

10:05 am Discussion

10:10 am **Daniel Grun**
Epidermal cancer stem cells drive formation of highly vascularized and aggressive tumors via a novel mechanism that requires VEGF-A binding to NRP-1 transmembrane receptor (Abstract #13)

10:25 am Discussion

10:30 am **Erik Martin**
Use of membrane-anchored serine protease-targeted anthrax toxin proteins to reduce tumor growth and metastasis (Abstract #16)

10:45 am Discussion

Poster Presentations I: Graduate Students, *Room 210*

10:45 am-11:45 am Poster Presentations and Networking

Lunch, *Room 349*

11:45 am-12:45 pm

Oral Session II: Postdoctoral, *Room 208*

Chair: **Nadire Duru, Ph.D.**

12:45 pm — **Katie Leonard, Ph.D.**
NME1 mediates a switch in expression of beta-integrin subunits that correlates with prolonged patient survival (Abstract #36)

1:00 pm — Discussion

1:05 pm — **Lorenzo Stramucci, Ph.D.**
MSCs prevent imatinib-induced apoptosis and induce quiescence of BCR-ABL+ cells through generation of PP2A-regulated signals (Abstract #42)

1:20 pm — Discussion

1:25 pm — **Kyu Lee Han, Ph.D.**
Semaphorin 4D induces myeloid derived suppressor cells in human head and neck squamous cell carcinoma (Abstract #32)

1:40 pm — Discussion

1:45 pm — **Palak Parekh, Ph.D.**
DUOX2- the key player for hyper-radiosensitivity in gastric cancer cells with low dose fractionation radiotherapy (Abstract #38)

2:00 pm — Discussion

Poster Presentations II: Postdoctoral Fellows and Other Attendees, *Room 210*

2:00 pm-3:00 pm — Poster Presentations and Networking

GCC Overview, *Room 208*

3:00 pm-3:15 pm — **Richard L. Eckert, Ph.D.**
John F.B. Weaver Distinguished Professor and Chair, Department of Biochemistry and Molecular Biology; Associate Director-Basic Sciences, The University of Maryland Marlene and Stewart Greenebaum Cancer Center

Keynote Presentation, *Room 208*

3:15 pm — Introduction: **Tyler Gable**

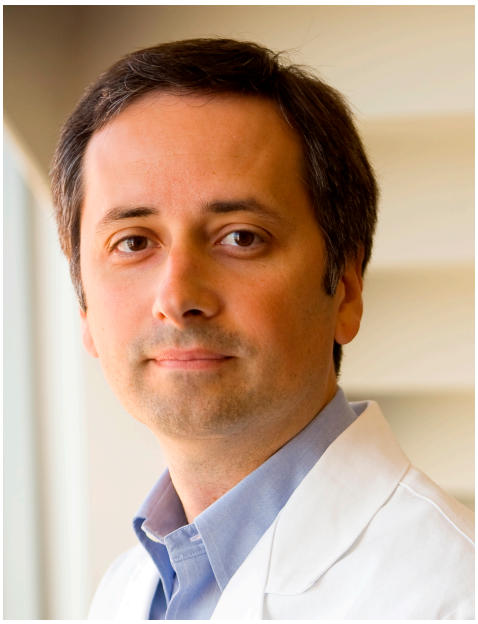
3:20 pm — **Victor E. Velculescu, M.D., Ph.D.**
Professor of Oncology and Pathology at the Johns Hopkins University Sidney Kimmel Cancer Center

"Liquid Biopsy Approaches for Detection and Characterization of Human Cancer"

Awards Ceremony, *Room 208*

4:20 pm-4:30 pm

Keynote Speaker



Dr. Victor E. Velculescu is Co-Director of Cancer Biology and Professor of Oncology and Pathology at the Johns Hopkins University Kimmel Cancer Center. His research interests are focused on genomic analyses of human cancer and using such information to understand the underlying biology of cancer and to identify new diagnostic and therapeutic approaches.

Dr. Velculescu is internationally known for his genomic discoveries in human cancer. He developed SAGE (serial analysis of gene expression) and used this method to perform the first transcriptome analysis in eukaryotic cells. Subsequently, he developed Digital Karyotyping for analysis of structural genomic alterations and together with his colleagues performed the first sequence analysis of the coding genome in human cancers, including breast, colorectal, brain, pancreatic, and ovarian cancers. These analyses identified a variety of genes not previously known to be involved in neoplasia, including the *PIK3CA* gene as one of the most highly mutated genes in human cancer. More recently, his group has developed PARE (personalized analysis of rearranged ends) for non-invasive liquid biopsy approaches for tumor detection and monitoring. These discoveries provide insights into the mechanistic features and pathways underlying human cancer and provide new opportunities for individualized diagnostic and therapeutic approaches.

Dr. Velculescu completed his B.S. at Stanford University and his M.D., Ph.D., and Postdoctoral Fellowship in Oncology at Johns Hopkins University. Dr. Velculescu is a member of the Board of Directors of AACR, has served as a member of scientific advisory boards of Basser Research Center at the University of Pennsylvania, the Starr Cancer Consortium, Quintiles, Helicos Biosciences, Inostics, and SoftGenetics, and is a co-founder of Personal Genome Diagnostics. He is the recipient of several awards for his work including the Grand Prize Winner of the Amersham/Pharmacia & *Science* Young Scientist Prize (1999), Judson Daland Prize of the American Philosophical Society (2008), the European Association of Cancer Research and *Carcinogenesis* Young Investigator Award (2008), the AACR Award for Outstanding Achievement in Cancer Research (2009), the Paul Marks Prize for Cancer Research (2011), and the AACR Team Science Awards for Pancreatic (2013) and Brain Cancer Research (2014).

Abstracts

Graduate Students:

1. **Identification and Characterization of S100B Targets in Melanoma**

Presenter: Milad Alasady Mentor: David Weber
Department of Biochemistry and Molecular Biology

S100B belongs to the S100 protein family, a large family consists of more than 20 members of intracellular and extracellular calcium-binding proteins. S100B exerts its function via interactions with many target proteins such as enzymes, transcription factors, and receptors to regulate proliferation, survival, cell growth, apoptosis, cell structure and shape, differentiation, migration, and invasion. To examine the role of S100B in melanoma, comparative RT2PCR gene arrays (Qiagen) were conducted on WM115 melanoma cell lines engineered to express low levels of S100B. The mRNA levels of 84 genes related to p53 pathway were evaluated. The mRNA of interleukin-6 (IL6) and hexokinase 2 (HK2) were highly elevated in the S100B knock-down WM115 cell line compared to the WM115 with high endogenous S100B levels. Conversely; the mRNA of BAI1 was greatly decreased in the knockdown of S100B. Western blotting analysis showed a positive correlation between protein and mRNA levels of IL6, HK2, and BAI1. To test whether extracellular S100B could reverse the effect of knocking down endogenous S100B, purified (>95%) recombinant S100B was added to the stable S100B knockdown WM115 cells. However, addition of recombinant S100B did not alter the levels of IL6, HK2, and BAI1. While the exact mechanism of this regulation has not been determined yet, this study identified novel targets of S100B, and thus calcium, signaling. The ultimate goal of this research is to identify mechanism(s) of how S100B promotes cell signaling in melanoma, discover S100B interacting partners, and to eventually develop therapeutic drugs to inhibit their interaction with S100B for use as a potential future anti-cancer therapeutics.

2. **The Role of TWEAK-Fn14 Signaling in Tumor Cell Metastasis**

Presenter: Cheryl Armstrong Mentor: Jeff Winkles
Department of Surgery

TWEAK and Fn14, a ligand and receptor pair belonging to the TNF and TNFR superfamilies, respectively, have a normal physiological role in tissue repair following acute injury. Fn14 expression, which is low in normal tissue and up-regulated upon acute injury, is also up-regulated in many solid tumor types. Additionally, TWEAK expression is detected in some solid tumor types. As higher Fn14 expression has been found in metastatic tissue compared to the corresponding primary tumor tissue, this suggests that TWEAK-Fn14 signaling may be important to the process of metastasis. I have used an immune-competent mouse model to test the hypothesis that TWEAK-Fn14 signal transduction in cancer cells promotes metastasis. Furthermore, I have used immune-deficient mouse models to determine that TWEAK-Fn14 signaling inhibits NK-cell mediated anti-tumor immunity. I am currently investigating the mechanism by which TWEAK-Fn14 signaling inhibits anti-tumor immunity and promotes the development of metastases.

3. **Expression of MT1-MMP in Head and Neck Squamous Cell Carcinoma is Regulated by Hypoxia and Semaphorin 4D**

Presenter: Amr Bugshan Mentor: John Basile
Department of Oncology and Diagnostic Sciences

Matrix metalloproteinases (MMPs) are a family of 19 soluble and 6 transmembrane proteins sharing the same catalytic domain that aid in cell migration through digestion of extracellular matrix (ECM) proteins and some non-matrix proteins as well. Membrane type 1 matrix metalloproteinase (MT1-MMP) is an integral membrane protein that is important in tumor growth, migration and invasion. It has the ability to degrade ECM, non-matrix proteins such as CD44 and integrin, and activate MMP2. The semaphorins are comprised of eight classes of membrane bound and secreted proteins that play

an essential role in axonal guidance and cell adhesion and migration during development, with the plexins and neuropilins acting as their main receptors. Semaphorin 4D (Sema4D), a membrane bound semaphorin, is highly expressed in malignancies such as head and neck squamous cell carcinoma (HNSCC) and is known to be pro-angiogenic, promoting the growth of blood vessels into a developing tumor by acting as a chemoattractant when bound to its receptor, Plexin-B1, on endothelial cells. Recently, it has been shown that MT1-MMP is necessary for the shedding of Sema4D from the cell surface to generate a soluble form that can form a chemotactic gradient necessary for attracting blood vessels. Our hypothesis is that tumor hypoxia causes an increase in Sema4D which acts in an autocrine and paracrine manner on tumor cells to induce the overexpression of MT1-MMP, which in turn cleaves Sema4D and increases availability to the tumor microenvironment to promote tumor-induced angiogenesis. Here we demonstrate through immunoblot and flow cytometry that MT1-MMP increases in HNSCC cells in a Sema4D and Plexin-B1-dependent manner in hypoxia. We also demonstrate that the Plexin-B1 downstream effectors RhoA and NF-κB are important in the regulation of cell surface MT1-MMP expression under hypoxic conditions. Based on our data, we conclude that Sema4D controls its own availability and therefore its own pro-angiogenic potential through autocrine/paracrine regulation of MT1-MMP.

4**. HDAC 9 and 11 Contribute to UV Resistance in Melanoma Cells**

Presenter: Elizabeth Chang Mentor: France Carrier
Department of Radiation Oncology

We have previously shown that the Histone Deacetylase Inhibitor (HDACi) trichostatin A (TSA) can sensitize cancer cells to UV radiation by facilitating the formation of Cyclobutane pyrimidine dimers (CPD) without increasing the levels of HDAC 1. Here we show that HDAC 9 and HDAC 11 are upregulated in response to UV radiation in a number of human melanoma cell lines. Both HDAC 9 and HDAC 11 have an hnRNP A18 signature motif in their 3'UTR. hnRNP A18 is a new regulator of protein translation that can confer growth advantages to tumor cells by specifically increasing the stability and translation of mRNA transcripts harboring its signature motif. Our data indicate that HDAC 9 and 11 levels correspond to hnRNP A18 level in response to UV radiation in several melanoma cell lines while hnRNP A18 and HDAC 9 and 11 are not upregulated in response to UV radiation in the normal human fibroblasts Malme cells or the normal melanocytes HEMa-LP. Moreover, clonogenic colony survival assays indicate that melanoma cells expressing higher hnRNP A18 endogenous levels are more resistant to UV radiation than cells expressing lower hnRNP A18 levels. In addition, down regulation of hnRNP A18 prevents HDAC 9 and 11 upregulation in response to UV radiation. Furthermore, overexpression of hnRNP A18 increases further the levels of HDAC 9 and 11 in response to UV radiation. These data suggest that upregulation of HDAC9 and 11 in response to hnRNP A18 activation contribute to UV resistance and may increase sensitization to UV-induced cell killing by HDACi.

5. **MARCH5 Regulates Mitochondrial Homeostasis and Cell Viability**

Presenter: Edward Cherok Mentor: Mariuz Karbowski
Department of Biochemistry and Molecular Biology

MYC oncogene is one of the most commonly amplified genes in breast cancers (BC), including the most clinically challenging subtype for therapeutic targeting, triple-negative breast cancers (TNBC). Deregulation of MYC activity and proteins is also associated with aggressive and poorly differentiated tumors. However, little is known about the role of MYC in development of genomic instability in these cancers. We have recently reported that TNBC cells upregulate components of alternative and error-prone form of non-homologous end-joining NHEJ repair (ALT NHEJ), such as DNA ligase III (LIG3) and PARP1, and are highly sensitive to its inhibitors. The aim of this study is to determine the mechanisms underlying ALT-NHEJ upregulation in TNBCs. Herein, we demonstrate that MYC plays a novel role in driving error-prone repair by ALT NHEJ. A significant decrease in PARP1 and LIG3 mRNA and protein is observed with siRNA-mediated knockdown (KD) of MYC in several MYC- dependent TNBC cells (n=3). Furthermore, overexpression of MYC cDNA leads to >2-fold increase in steady- state levels of PARP1 and LIG3. Next, we assessed whether MYC

expression affects the functional outcome of DSB repair via ALT NHEJ. Notably, MYC KD led to an increase in fidelity of NHEJ repair in TNBCs (in vitro) and a decrease in repair via ALT NHEJ (in vivo). In vivo NHEJ assays on cells released from synchronization at S- and G2/M phases of cell cycle reveal that DSB repair errors in the plasmid are introduced mainly in the S-phase, where ALT NHEJ is more predominant. Through chromatin immunoprecipitation (ChIP) analysis, we also demonstrate direct binding of MYC on the promoters of both PARP1 and LIG3. Our findings demonstrate a novel role of MYC in driving error-prone DSB repair by ALT NHEJ pathway, which could have important implications in genomic instability, disease progression and the development of novel therapies in TNBCs.

6. **Combination Treatment of PARP inhibitor, BMN 673 and DNMT Inhibitor, Azacytidine: A Potential Therapy for BRCA Mutant and Wild Type, Triple Negative Breast Cancers?**

Presenter: Khadiza Chowdhury Mentor: Feyruz Rassool
Department of Radiation Oncology

Poly-ADP-ribose polymerase inhibitors (PARPis) represent one of the most exciting recent developments in cancer therapy and have shown efficacy in a minority (5-10%) of triple negative (estrogen, progesterone and herceptin receptor negative) breast cancers (TNBCs) with inherited mutations in breast cancer genes BRCA1/2. In PARPi clinical trials, approximately of 60% BRCA mutant patients showed responses, while a significant proportion did not. In addition, sporadic TNBCs with intact BRCA1/2 have failed PARPi therapy. This suggests the urgent need for additional novel therapies for breast cancer. Our studies are underpinned by 4 important findings: (1) the development of a new class of potent PARPis exemplified by BMN 673, (2) the mechanism of cytotoxicity of PARPis, including BMN 673, is attributed to its ability to trap PARP–DNA complexes in chromatin, (3) unpublished findings by our group that PARP1 interacts with epigenetic factors such as DNMT1 in a high molecular weight complex on chromatin in response to DNA damage and (4) DNMT inhibitors (DNMTis) such as FDA approved Azacytidine (AZA) covalently trap DNMTs in DNA as their mechanism of action. Therefore, the interaction of PARP1 and DNMT1 suggest a therapeutic opportunity for combining PARPis and DNMTis in an effort to enhance the effects of PARPi therapy. We first set out to determine whether a combination of these two drugs would enhance trapping of PARP-DNA complexes in chromatin, leading to increased cytotoxicity of TNBC. We treated BRCA wild type, MDA-MB-231 and BRCA mutant, SUM149PT with BMN 673 (10nM) or AZA (150 or 250nM respectively) alone, or the two drugs in combination, and measured PARP1 trapping in chromatin extracts following induction of DNA damage by radiation (2Gy) after 72 or 48hrs respectively. As expected, BMN 673 treatment leads to PARP1 trapping as determined by western blotting of proteins from chromatin extracts. Interestingly, treatment with AZA treatment alone also leads to the presence of PARP1 in chromatin. Importantly, AZA in combination with BMN 673 treatment shows an at least a 2-fold increase in PARP1 trapping, compared to the individual drug treatments. To determine whether PARP1 trapping correlates with cytotoxicity and cell death, BRCA wild type TNBC lines MDA-MB-231 and MDA-MB-468 and BRCA mutant TNBC lines SUM149PT and HCC1937 cells were treated with the drugs alone and in combination and placed in colony forming assays. Compared with single drug treatments, the combination treatment showed a significant ($p<0.05$) decrease in colony formation in both BRCA wild type and mutant cell lines indicating BMN 673 could potentially be used in combination with AZA to improve treatment of TNBCs. In vivo studies in BRCA mutant SUM149PT xenograft mouse model also showed reduction in tumor growth and increased survival in combination treatment compared to the single and vehicle treatments.

7. **Triple SILAC-based Glycoproteomic Approach Identifies KRAS as a Positive Regulator of CREG1 in Human Lung Adenocarcinoma Cells**

Presenter: David Clark Mentor: Li Mao
Department of Oncology and Diagnostic Sciences

Protein glycosylation plays a fundamental role in a multitude of biological processes, and the associated aberrant expression of glycoproteins in cancer has made them attractive targets as biomarkers and therapeutic targets. In this study, we examined differentially expressed glycoproteins in cell lines derived from three different states of lung tumorigenesis: an immortalized bronchial epithelial cell (HBE) line, a non-small cell lung cancer (NSCLC) cell line harboring a Kirsten rat sarcoma viral oncogene homolog (KRAS) activation mutation and a NSCLC cell line harboring an epidermal growth factor receptor (EGFR) activation deletion. Using a Triple SILAC proteomic quantification strategy paired with hydrazide chemistry N-linked glycopeptide enrichment, we quantified 118 glycopeptides derived from 82 glycoproteins in the three cell lines. Proteomic profiling revealed 27 glycopeptides overexpressed in both NSCLC cell lines, 6 glycopeptides overexpressed only in the EGFR mutant cells and 19 glycopeptides overexpressed only in the KRAS mutant cells. Further investigation of a panel of NSCLC cell lines found that Cellular repressor of E1A-stimulated genes (CREG1) overexpression was closely correlated with KRAS mutation status in lung adenocarcinoma cells and could be down-regulated by inhibition of KRAS expression. Our results indicate that CREG1 is a down-stream effector of KRAS in lung adenocarcinoma cells and a novel candidate biomarker for KRAS mutant tumors.

8. **Modification of Thymine DNA Glycosylase by Small Ubiquitin-Like Modifiers Is Efficient but Not Selective for the Enzyme-Product Complex**

Presenter: Christopher Coey Mentor: Alex Drohat
Department of Biochemistry and Molecular Biology

Thymine DNA glycosylase (TDG) initiates the repair of G•T mismatches that arise by deamination of 5 methylcytosine (mC), and it excises 5 formylcytosine and 5-carboxylcytosine, oxidized forms of mC. TDG functions in active DNA demethylation and is essential for embryonic development. TDG forms a tight enzyme-product complex with abasic DNA, which severely impedes enzymatic turnover. Modification of TDG by small ubiquitin-like modifier (SUMO) proteins weakens its binding to abasic DNA. It was proposed that sumoylation of product-bound TDG regulates product release, with SUMO conjugation and deconjugation needed for each catalytic cycle, but this model remains unsubstantiated. We examined the efficiency and specificity of TDG sumoylation using in vitro assays with purified E1 and E2 enzymes. Under multiple-turnover conditions, TDG was modified efficiently by SUMO-1 and SUMO-2, but SUMO-2 modification was incomplete. Remarkably, we observed similar modification rates for free and DNA-bound TDG. To examine the conjugation step more directly, we determined the modification rate (kobs) using preformed E2~SUMO-1 thioester. The hyperbolic dependence of kobs on TDG concentration gives kobsmax = 1.6 min-1 and K1/2 = 0.55 μM. This result suggests that E2~SUMO-1 has higher affinity for TDG than for RanGAP1 and p53 (peptide). Modification by E2~SUMO-1 is slowed by 30% and 50% when TDG is bound to abasic and nonspecific DNA, respectively. Although E2~SUMO-1 exhibits no specificity for product-bound TDG, the conjugation efficiency leaves open the possibility that sumoylation could stimulate product release in vivo. This and other implications for the biological role and mechanism of TDG sumoylation are discussed.

9. **The Role of Testisin and PAR-2 Signaling in Metastatic Ovarian Cancer**

Presenter: Greg Conway Mentor: Toni Antalis
Department of Physiology

Ovarian cancer is the most lethal gynecological malignancy with a 5-year case fatality rate over 50%; it will affect one in every 69 women. Due to nonspecific symptoms and a limited ability to detect the tumors, patients often present with advanced, metastatic disease and have a poor prognosis. Neovascularization of tumors enables growth beyond microscopic size, and ovarian cancer relies on changes to a number of angiogenic factors such as vascular endothelial growth factor-A (VEGFA), angiopoeitin1 (ANG1) and angiopoeitin2 (ANG2) to promote tumor growth and metastasis. At this time, a detailed understanding of the molecular mechanisms involved in VEGFA, ANG1 and ANG2 production by ovarian tumor cells and the underlying angiogenic processes that control the dissemination of ovarian cancer are not well understood. The G-protein coupled receptor protease activated receptor-2 (PAR-2), is overexpressed in ovarian cancer. Recently, we identified PAR-2 as the only known physiological substrate of the membrane anchored serine protease testisin. Expression of testisin has been shown to promote angiogenic responses in mice and importantly, while testisin is overexpressed in ovarian cancer and correlated with advanced disease, no testisin expression is detected in the normal ovary or fallopian tubes. We are testing the hypothesis that VEGFA and ANG1 production by ovarian tumor cells is caused by aberrant, constitutive expression of the serine protease, testisin and PAR-2 signaling. We have examined the role of testisin in VEGFA and ANGI production by transiently transfecting testisin into the ovarian cancer cell line SKOV3 and subsequently measured mRNA and protein expression via qPCR and ELISA, respectively. Transfection of testisin resulted in an increase in VEGFA mRNA and protein and a concomitant decrease in ANG1 mRNA in SKOV3 cells. We next silenced testisin via shRNA in the ovarian cancer cell line NCI/ADR-Res and in HeLa cells. Knockdown of testisin led to a loss of VEGFA mRNA and an increase in ANG1 mRNA in both HeLa and NCI/ADR-Res cell lines. To begin to examine the role of PAR-2 in VEGFA and ANG1 production we treated three ovarian cancer cell lines (ES-2, SKOV3, OVCAR3) with a PAR-2 activating peptide (SLIGKV-NH2) and measured changes in VEGFA protein release and ANG1 mRNA. Treatment of all three ovarian cancer cell lines with PAR-2 activating peptide lead to an increase in VEGFA protein release as measured by ELISA and decreased ANG1 mRNA expression by qPCR. The similar effects of changes in testisin and PAR-2 signaling on downstream VEGFA and ANG1 supports the hypothesis that testisin and PAR-2 are acting together in a signaling cascade. Future studies will aim to confirm these findings and elucidate whether testisin activates VEGFA and ANG1 directly through PAR2 signaling. Importantly, as testisin has not been found to be expressed in the normal ovary but is highly expressed in ovarian cancers, testisin is a unique and attractive therapeutic target for ovarian cancer and potential novel biomarker to direct therapy.

10. **The Pim Kinase Inhibitor AZD1208 Sensitizes Acute Myeloid Leukemia Cells with Fms-like Tyrosine Kinase 3 Internal Tandem Duplication (FLT3-ITD) to Cytotoxic Effects of Chemotherapy Drugs**

Presenter: Kshama Doshi Mentor: Maria Baer
Department of Medicine

Purpose: Internal tandem duplication (ITD) in the juxtamembrane domain of the fms-like tyrosine kinase 3 (FLT3) receptor is present in leukemia cells of approximately 30% of acute myeloid leukemia (AML) patients. These patients have a high remission rate, but short relapse-free and overall survival. Pim-1 kinase, a pro-survival oncogene, is transcriptionally upregulated downstream of FLT3-ITD and promotes FLT3-ITD signaling via a positive feedback loop. We proposed that Pim kinase inhibitors would enhance the cytotoxic effects of chemotherapy drugs in AML cell lines and patient samples with FLT3-ITD. Methods: AZD1208, a pan-Pim (Pim-1, Pim-2 and Pim-3) kinase inhibitor, was kindly provided by AstraZeneca. FLT3-ITD cell lines included MV4-11, MOLM-14 and Ba/F3-ITD. The topoisomerase 1 and 2 inhibitors daunorubicin, mitoxantrone, etoposide and topotecan and the nucleoside analog cytarabine were studied at their IC50 concentrations for each cell line. Apoptosis was measured by flow cytometric analysis of Annexin V/propidium iodide (PI) staining, percent cells in sub-G1 cell cycle phase using PI staining and mitochondrial membrane potential (MMP) using JC-1 dye, as well as flow cytometric and western blot measurements of cleaved poly (ADP-ribose)

polymerase (PARP) and procaspase-3. Results: Co-treatment with 1μM AZD1208 and daunorubicin induced significantly more apoptosis than daunorubicin or AZD1208 alone (e.g., 65% vs. 25% and 7% in Ba/F3-ITD) in FLT3-ITD cell lines. At least two-fold increase in apoptosis was also seen when AZD1208 was combined with mitoxantrone, etoposide or topotecan, compared with each drug alone, but AZD1208 did not increase apoptosis induced by cytarabine. Chemosensitization of FLT3-ITD cells with the combination treatment was confirmed by a higher percentage of sub-G1 cells (e.g., 33% for AZD1208 and daunorubicin combination vs. 17% for daunorubicin alone in Ba/F3-ITD), more rapid loss of MMP and increased time-dependent cleavage of PARP and procaspase-3. Apoptosis was significantly ($P<0.0001$) rescued by co-treatment with the pan-caspase inhibitor z-VAD. Cell lines with wild-type FLT3 were less sensitive to combined AZD1208 and chemotherapy-induced apoptosis. Finally, increased cytotoxicity of daunorubicin in the presence of AZD1208 was also seen in FLT3-ITD AML patient marrow samples, but not remission marrow samples. Conclusion: Our work supports clinical applicability of combining a Pim kinase inhibitor with chemotherapy to treat AML with FLT3-ITD. Ongoing work is directed toward determining the molecular mechanisms of sensitization of FLT3-ITD cells to chemotherapy by Pim kinase inhibition.

11. **Transglutaminase 2 is Necessary for Cancer Stem Cell Survival in Squamous Cell Carcinoma**

Presenter: Matthew Fisher Mentor: Richard Eckert
Department of Biochemistry and Molecular Biology

Transglutaminases (TG) comprise a family of multifunctional proteins involved in regulation of cell survival and differentiation. Among these protein, type II transglutaminase (TG2) is the most widely distributed. TG2 functions as a calcium-dependent protein crosslinking protein, as a GTP binding protein, as a protein disulfide isoermase, and as a serine/threonine kinase. TG2 has been described as an important factor in cancer cell survival, and is increased in a numerous cancers. Levels are highly elevated in advanced disease and its expression also confers resistant to cytotoxic chemotherapy. In the present study, we demonstrate that TG2 is necessary for survival of epidermal cancer stem cells (ECS cells). We show that TG2 is highly enriched in ECS cells and expression is associated with high levels of other known stem cell markers including Oct4, Nanog, Sox2 and Ezh2. Expression is also associated with expression of EMT markers including snail, slug, twist, N-cadherin and vimentin. Knockdown of TG2 reduces ECS cell survival, migration, invasion and spheroid forming ability. Treatment with NC9, a TG2 specific inhibitor, also reduces spheroid formation and ECS cell migration and invasion. Of particular interest, TG2 is capable of inducing the stem and EMT phenotype independent of the crosslinking function. This implicates that the GTP binding function of TG2 is of importance in tumor progression. These studies identify TG2 as an important cancer therapy target in ECS cells.

12. **A Novel Cdc25A Localization Domain Imparts Graduated Nuclear Localization**

Presenter: Tyler Gable Mentor: Li Mao
Department of Oncology and Diagnostic Sciences

The Cdc25A phosphatase regulates cell cycle by activation of CDK/Cyclin complexes in nuclei, namely CDK2/Cycling-E and CDK1/Cyclin-B in S-phase and G2/M phase respectively. Over expression (oxCdc25A) causes dysregulation of cell cycle checkpoints and is associated with poorer prognosis in many cancers where oxCdc25A is observed. Thus, understanding the mechanism of nuclear localization is vital to inhibiting its oncogenic influence. Work in our lab has identified the existence of a previously unknown localization domain. Using confocal microscopy, I now define the role of a non-classical Nuclear Localization Domain (ncNLD) which functions in concert with the Nuclear Export Signal (NES) and classical Nuclear Localization Signal (cNLS) to fully localize Cdc25A to nuclei. This novel domain was also found to impart a graduated localization to the nucleus independent of the NES and cNLS. Taken together, these domains represent potential mechanisms by which failed cell cycle checkpoint arrest induced by oxCdc25A can be reversed or reduced. Efforts to screen for drug compounds which specifically inhibit localization of Cdc25A are underway. It is hopeful that therapeutically reducing nuclear Cdc25A may serve the dual purpose of restoring tumor sensitivity to ionizing radiation and inducing apoptosis with restored DSB repair capacity in diseased tissues while simultaneously promoting radiation resistance in tissues without oxCdc25A.

13. **Epidermal Cancer Stem Cells Drive Formation of Highly Vascularized and Aggressive Tumors via a Novel Mechanism that Requires VEGF-A Binding to NRP-1 Transmembrane Receptor**

Presenter: Daniel Grun Mentor: Richard Eckert
Department of Biochemistry and Molecular Biology

Epidermal cancer stem cells (ECS cells) comprise a limited subpopulation (0.15%) of cells present in epidermal squamous cell carcinoma tumors. In contrast to non-stem cancer cells, ECS cells from aggressive, invasive, and highly vascularized/CD31 vascularization marker-positive tumors that are four times larger than the small, circumscribed, non-vascularized tumors formed by non-stem cancer cells. This suggests that ECS cells are highly dangerous cells that must be contained to reduce skin cancer tumor growth and recurrence. Our goal in this study is to understand the mechanism of aggressive growth and vascularization, and to identify therapy options. We hypothesize that ECS cells produce angiogenesis-related agents that stimulate tumor vascularization and also stimulate ECS cell survival. We find that ECS cells produced enriched levels of pro-angiogenic factors, including VEGF-A, HIF-1-alpha and MMP9, as compared to tumors derived from non-stem cancer cells. In addition, anti-angiogenic factor (thrombospondin-1, endothelin-1, serpin-B5) production is reduced. VEGF-A knockdown or treatment with VEGFA-inactivating antibody reduce ECS cell spheroid formation, and cell migration and invasion, indicating that VEGF-A is required for these responses. Moreover, tumor xenograft experiments show that treatment with bevacizumab, an anti-VEGF-A therapy being used in patients, suppresses in vivo tumor formation. Surprisingly, the traditional VEGF-A mechanism of action, involving interaction with VEGFR1 or VEGFR2, does not mediate VEGF-A action in these cells, as VEGFR1 and VEGFR2 are not expressed in ECS cells and treatment with siRNA to these receptors has no impact on VEGF-A dependent events. Instead, we observed that knockdown of NRP1, a VEGFR2 co-receptor and PDZ domain protein, attenuates VEGF-A dependent ECS cell survival, migration and invasion. Moreover, an NRP1 inhibitor (EG00229) reduces tumor formation. Based on these and other findings, we propose a novel mechanism of ECS cell-associated tumor vascularization that involves VEGF-A binding to NRP1 to stimulate intracellular events to promote ECS cell survival, migration and invasion leading to formation of highly vascularized and aggressive tumors. Moreover, our findings suggest that VEGF-A and NRP1 are important candidate therapy targets.

14. **Multiple Drug resistance-associated protein 4 (MRP4) May Contribute to Breast Cancer Metastasis by Exporting the COX-2 Product, PGE2**

Presenter: Tyler Kochel Mentor: Amy Fulton
Department of Pathology

Cyclooxygenase-2 (COX-2) and its enzymatic product, prostaglandin E2 (PGE2), are elevated in breast cancer and are associated with a poor prognosis and increased metastatic potential. PGE2 initiates various signaling pathways upon binding to each of four cognate EP receptors. We have previously shown that PGE2 signaling through the EP4 receptor increases metastatic potential and supports the survival of breast cancer stem-like cells. Multiple drug resistance-associated protein 4 (MRP4) is responsible for the active export of PGE2 from cells, while the prostaglandin transporter (PGT) imports PGE2 for 15-hydroxyprostaglandin dehydrogenase (15-PGDH)-mediated degradation. The role of neither MRP4 nor PGT has been investigated in breast cancer progression. The purpose of this study is to elucidate the role of MRP4 in PGE2 signaling in breast cancer progression. We hypothesize that elevated expression of MRP4 would cause increased PGE2 export and receptor-mediated signaling and, therefore, enhance metastatic potential, tumor progression, and support breast cancer stem-like cells. We examined MRP4 gene expression data from multiple breast cancer datasets using Oncomine. We found higher expression of ABCC4 in breast cancer versus normal breast. MRP4 was elevated in invasive (IDC) versus localized (DCIS) lesions and elevated in the majority of basal-type breast cancer. To investigate the role of MRP4 further, we examined MRP4 mRNA and protein expression in cell lines representing several molecular subtypes and metastatic capacities. Normal mammary epithelium (MCF10A), luminal (MCF7, T47D), basal (MDA-MB-231, MDA-MB-468, MDA-MB-436, BT549), and Her2-enriched (SKBR3) cell lines were evaluated. As in the primary sample data, MRP4 mRNA and protein expression are elevated in basal and Her2 enriched cell lines (231, 436, BT549, SKBR3) while expression of PGT mRNA and protein is decreased in these cells when compared to cells with lower

metastatic potential. This inverse relationship between MRP4 and PGT should lead to higher concentrations of extracellular PGE2 in the tumor microenvironment. We evaluated MRP4 activity by measuring PGE2 export from cells via enzyme immunoassay or resistance to the cytotoxic compound 6-mercaptopurine (6-MP), two substrates of MRP4. Pharmacologic inhibition of MRP4 with MK571 (MRP antagonist) results in decreased efflux of PGE2 and increased sensitivity to 6-MP as expected. Likewise, genetic suppression by RNAi results in lower levels of PGE2 exported from cells and increased sensitivity to 6-MP, confirming altered MRP4 activity. Conversely, ectopic MRP4 overexpression increases PGE2 export. These data support the hypothesis that MRP4 is a critical member of the PGE2 signaling pathway that leads to high extracellular PGE2 and increased PGE2 signaling, implicating MRP4 as a possible therapeutic target in this oncogenic pathway.

15. **MNK Driven mRNA Translation in DLBCL**

Presenter: Ari Landon Mentor: Ron Gartenhaus
Department of Medicine

Dysregulation of mRNA translation alters cellular phenotypes by altering the level of select proteins. These changes in protein levels can lead to cancer initiation, maintenance and progression. Cap-dependent translation is the primary mechanism of mRNA translation in eukaryotic cells. Cap-dependent translation is initiated by eIF4E1 binding to the 7-methyl-guanosine-cap (cap). The conventional translation initiation complex is known as eIF4F. eIF4F consists of eIF4A, eIF4G and eIF4E. eIF4E binds the cap structure in the mRNA functioning as a bridge between mRNA and the ribosome. MNK kinases are responsible for phosphorylating eIF4E1. Interestingly we found that knocking down MNK kinases translationally represses total eIF4E1 specifically in B-cells. This novel impact on total eIF4E1 demonstrates a potential for MNKs to directly impact a large subset of oncogenes in a B-cell specific manner. Inhibiting MNK kinase activity and subsequent eIF4E1 phosphorylation caused an increase in eIF4E3 expression. eIF4E3 is another member of the eIF4E family that has been shown to bind the cap by an atypical mechanism. We explored the biological function of eIF4E3 in a diffuse large B-cell lymphoma model (DLBCL). The increase in total eIF4E3 leads to more eIF4E3 binding to the cap in place of eIF4E1. We find that eIF4E3 also physically associates with eIF4A and eIF4G forming a novel eIF4F complex. We interrogated the functional aspects of eIF4E3-mediated translation by analyzing the translatome and transcriptome of eIF4E3 expressing cells. eIF4E3 and eIF4E1 mutually regulate a high fraction of total mRNA transcripts while both family members exclusively modulate translation of select messages. Interestingly, we identified selective motif enrichments in the 5'UTR that may facilitate transcript selection by eIF4E1 or eIF4E3. These findings imply alternative ribosomal complexes are able to selectively translate mRNA via transcript recognition. The dual roles of MNK kinases in both modulating eIF4E1 phosphorylation as well as total eIF4E1 protein levels highlight MNK as a powerful oncogenic driver.

16. **Use of Membrane-anchored Serine Protease-targeted Anthrax Toxin Proteins to Reduce Tumor Growth and Metastasis**

Presenter: Erik Martin Mentor: Toni Antalis
Department of Physiology

Anthrax toxin is a three-component toxin secreted by Bacillus anthracis. The toxin's mechanism of action requires one of its components, protective antigen, to be proteolytically activated in order to deliver the other components, lethal factor and edema factor, into the cytosol to induce cytotoxicity. Typically, cleavage and activation of protective antigen is catalyzed by the protease furin on the cell surface. Protective antigen has previously been engineered to be activated by MMPs and/or uPA as an alternative to furin, and these engineered protective antigen proteins have been used in conjunction with other anthrax toxin components, as tumor-selective, protease-activated, prodrug-like reagents capable of achieving tumor cell cytotoxicity in vitro and in vivo. In an attempt to develop a protease-activated prodrug strategy to target the proteolytic activities of a group of proteases known as the membrane-anchored serine proteases, which are tethered to surfaces of cells via transmembrane domains or GPI-linkages and often found over-expressed on many types of tumor cells, we have created novel anthrax toxin protective antigen proteins containing activation sequences

predicted to be cleaved by membrane-anchored serine proteases. We found that these engineered protective antigen proteins are able to be cleaved by membrane-anchored serine proteases in vitro and in cell culture. We also show that they are able to induce tumor-cell cytotoxicity in cell culture and xenograft tumor growth inhibition in vivo, when combined with other engineered anthrax toxin components. We are currently investigating the abilities of the novel protective antigen proteins to be used in vivo to reduce ovarian tumor burden in a metastatic xenograft tumor model.

17. **Targeting Telomere Extension in Head and Neck Cancer**

Presenter: Alex Meltzer Mentor: Michal Zalzman
Department of Biochemistry and Molecular Biology

Telomeres are DNA repeat sequences at the ends of chromosomes that protect the chromosomes as well as serve as a biological clock. As cells divide, telomeres shorten until they reach a critically short length, at which point they signal the cell to stop replicating. The enzyme telomerase has been proposed to maintain telomere length and immortality in cancer cells, justifying it as a therapeutic target of cancer. However, because telomerase is expressed in non cancerous cells and telomerase inhibitors are associated with toxicities, a better target is needed. We recently identified a novel, telomerase-independent mechanism that is necessary and sufficient for telomere maintenance of mouse embryonic stem (ES) cells activated by the gene ZSCAN4. We now show that ZSCAN4 is reactivated in human head and neck squamous cell carcinoma, and its loss results in gradual telomere shortening, corresponding with in an induction of cellular senescence followed by cell death. Our current work focuses on the function of ZSCAN4 and its associated mechanism in head and neck squamous cell carcinoma. Our findings further elucidate the mechanism by which ZSCAN4 mediates immortality in cancer, and may ultimately lead to novel targets for cancer therapy.

18. **Combination of DNA Methyltransferase and PARP Inhibitors as a Novel Therapy Strategy for Poor Prognosis Acute Myeloid Leukemia**

Presenter: Nidal Muvarak Mentor: Feyruz Rassool
Department of Radiation Oncology

We present here strong preclinical data for a novel, mechanistically-based, combinatorial approach to using DNA methyltransferase inhibitors (DNMTi's), such as decitabine (DAC) and 5-Azacytidine (AzaC), with PARP inhibitors (PARPi's) as a treatment strategy for acute myelogenous leukemias (AML). AzaC and DAC alone show efficacy in AML, but combinatorial approaches will be required to maximize therapeutic responses. PARPi's have not been well studied as agents for this disease. The mechanistic rationale for our approach is based upon: 1) data from our group and others showing DNMT and PARP co-reside in DNA damage induced protein complexes; 2) the fact that AzaC and DAC trap DNMT's into DNA via their mechanism of action, led us to hypothesize that these drugs might also increase PARP trapping at DNA damage sites 3) the cytotoxicity of clinically available PARPi's, and especially the most potent ones, appears to correlate with degree of trapping of PARP1 at DNA damage sites in chromatin. We first find that in cultured human AML cells, the DNMTi's (10 to 20 nM DAC) and PARPi's (1 to 10 nM BMN 673) alone trap PARP into chromatin and this effect is enhanced when the drugs are combined. Concomitant with this, the combined doses strongly induce double strand breaks (DSBs), thereby increasing cytotoxic DNA damage. In colony forming assays of both cultured (N=4) and primary AML cells (N=9), a combination of the DNMTi's and PARPi's strongly decreased colony survival compared to each of the agents alone. Interestingly those cell lines and primary samples expressing poor prognostic FLT3/ITD (Fms-like tyrosine kinase 3 internal tandem duplication) mutations, were particularly sensitive to the combination treatment. Based on all the above results, we developed an in vivo treatment model using human FLT3/ITD-positive MV411-luc xenografts in immunocompromised mice. As opposed to mock treatment and AzaC or BMN673 alone, the combined drug treatment significantly decreases leukemia burden, as measured by bioluminescence imaging, peripheral blood blast counts and spleen weights. Our data suggest a novel use of both DNMTi's and PARPi's in a compelling therapeutic strategy for poor prognosis AML.

19. **Identifying Effectors in NME1-mediated Metastasis Suppression in Malignant Melanoma**

Presenter: Nidhi Pamidimukkala Mentor: David Kaetzel
Department of Biochemistry and Molecular Biology

NME1 was the first described metastasis suppressor, discovered through its reduced expression in metastatic melanoma cells compared to non-metastatic counterparts. Despite extensive research over the years, the mechanism by which NME1 exhibits its metastasis suppressing activity is not well understood. NME1 has two well-described enzymatic activities which may contribute to its metastasis suppressor function: a nucleoside diphosphate kinase (NDPK) activity that mediates the reciprocal transfer of a phosphate from a nucleoside triphosphate to a nucleoside diphosphate, and a 3′ to 5′ exonuclease activity which removes 3′ overhanging bases on DNA. Our lab identified the 3′ to 5′ exonuclease activity, and likely the NDPK activity, to be necessary for metastasis suppression. NME1 has further been shown to regulate transcription, which may also contribute to its metastasis suppressor function. The lab has shown that NME1 binds to the promoter region and suppresses transcription of the platelet derived growth factor A (PDGF-A) oncogene. In breast cancer, NME1 was shown to regulate expression of several genes, with suppression of LPA receptor, EDG2, largely contributing to its metastasis suppressor function. These results strongly suggest that NME1 regulates expression of target genes that modulate the metastatic phenotype of malignant cells.

20**. Defining the Role of Testisin, a Membrane Anchored Serine Protease, in the Endothelium and Angiogenesis**

Presenter: R.J. Peroutka Mentor: Toni Antalis
Department of Physiology

Testisin (PRSS21), an extracellular GPI anchored serine protease, exhibits a very limited tissue distribution wherein it is most highly expressed in the testis. It has been found however that testisin mRNA is also expressed in human microvascular endothelial cells (HMVECs) undergoing capillary morphogenesis, but not in macrovascular human umbilical vein endothelial cells (HUVECs). In preliminary in vivo studies, testisin knockout mice displayed delayed angiogenesis in an aortic ring outgrowth assay and also in the corpus luteum following super-ovulation. Although the mechanism behind these observations is not known, PAR-2 receptor signaling has been demonstrated to significantly promote angiogenesis, and recently we have shown PAR-2 as a substrate for testisin cleavage and activation. We therefore hypothesize that testisin, transiently expressed on capillary endothelial cells, plays a functional role in endothelial cell biology, specifically capillary formation, and that this activity is at least in part mediated through PAR-2 activation and subsequent proangiogenic signaling. Although it has been demonstrated that targeting testisin expression by siRNA knock down can increase apoptosis and reduce growth in tumor cell lines, the effect of modulating testisin expression, or activity, in endothelial cells and its consequence on angiogenesis is not known. In an effort to better understand testisin activity and its role in endothelial cells we have initially produced recombinant testisin proteins, as there is no commercial source. Recombinant testisin was expressed in E. coli as insoluble inclusions, refolded and purified. We will use recombinant protein to study testisin's biochemical characteristics such as zymogen activation, SERPIN inhibition, specific activity, activation of endogenous PAR-2 signaling and possibly protein structure. Concurrent with enzymatic studies, we have also produced and purified several anti-testisin monoclonal antibodies and investigated their performance in applications such as immunohistochemistry, immunofluorescence, western blotting and immunoprecipitation. We are currently investigating how reducing the expression of testisin, or blocking the activation of PAR-2 signaling, impacts both micro- and macrovascular cell phenotypes using in vitro angiogenesis models.

21. **The Role of ZSCAN4 in HNSCC Tumor Development**

Presenter: Benjamin Portney Mentor: Michal Zalzman
Department of Biochemistry and Molecular Biology

Cancer cells are able to bypass normal cellular checkpoints intended to regulate cell cycle. One of these checkpoints is initiated by the shortening of repetitive nucleotide sequences at the end of chromosomes called telomeres. Telomeres shorten with every round of replication, ultimately reaching a critically short length and initiating a cellular checkpoint that halts cell division. However, cancer cells are able to reverse telomere shortening, leading to cellular immortality. We previously reported mouse ES cells employ a novel mechanism for telomere regulation induced by the activity of the gene ZSCAN4. ZSCAN4 is essential for immortality and long-term culture of mouse ES cells. However, little is known about the activity of the human ZSCAN4 protein. Like in mice, human ZSCAN4 is expressed in pre-implantation embryos and is not expressed in normal adult tissues. In this work we study the function of the human ZSCAN4 in head and neck squamous cell carcinoma (HNSCC). To study the effect of ZSCAN4 deficiency on tumor development in vivo we used a xenograft model in the NSG (NOD/SCID/IL2Rγ-/-) mice. Our current findings suggest ZSCAN4 is essential for tumor development in vivo.

22. **The Microbiome of Cancer as Seen Through the Lens of Sequence Data**

Presenter: Kelly Robinson Mentor: Julie Dunning Hotopp
Department of Microbiology and Immunology

There are 10 times more bacterial cells in the human body than human cells and various bacteria are known to influence carcinogenesis. Therefore we sought to investigate if whole genome and whole transcriptome sequencing data generated at UMB and through large public cancer genome efforts, like The Cancer Genome Atlas (TCGA), could be used to examine the microbial composition of tumors. The Burrows-Wheeler Aligner (BWA) was used to align the Illumina paired-end sequencing data to the human reference genome and all complete bacterial genomes in the RefSeq database. Reads were filtered for low complexity and duplicates and then assigned an Operational Taxonomic Unit (OTU). Through careful consideration of all of the bacterial taxa present in the cancer types investigated, we found an increased abundance of bacteria in some cancers, such as Pseudomonas spp. and Helicobacter pylori in stomach adenocarcinoma samples. Further investigation prompted us to conclude that some bacterial read pairs may be due to contamination from sample preparation or sequencing center batch effects. Upon completion of this analysis, we determined that it is possible to identify bacteria in human tumor samples. However, care must be taken to assess bacteria that arrived in the sample via various forms of contamination. In the future, a comprehensive approach should be used when evaluating the microbiome of tumor samples via sequencing data. The methods used in this study are valid and useful for identifying the presence of bacteria in human samples, but may not be an exhaustive picture. More weight should be given to this approach in the future when bacterial associations with diseases are suspected.

23. **The mRNA-destabilizing Protein Tristetraprolin Suppresses Tumorigenic Phenotypes in a Triple Negative Breast Cancer Cell Model via a Non-canonical Mechanism**

Presenter: Christina Ross Mentor: Gerald Wilson
Department of Biochemistry and Molecular Biology

The purpose of this study was to define mechanisms by which the mRNA-destabilizing factor tristetraprolin (TTP) impacts tumorigenic phenotypes in an aggressive model of metastatic breast cancer. Widespread repression of TTP levels in human tumors and cancer cell lines relative to non-transformed tissues suggests that TTP may function as a tumor suppressor in diverse neoplastic contexts, and low TTP expression is a negative prognostic indicator in breast cancer. TTP is a tandem zinc finger protein that binds to AU-rich elements (AREs) and targets their associated mRNAs for degradation. AREs are potent cis-acting determinants of cytoplasmic mRNA turnover in mammalian cells, and are essential for limiting cellular production of many clinically important gene products including regulators of inflammation,

cell proliferation, and apoptosis. In this study, we show that restoration of TTP levels attenuates several tumorigenic phenotypes in the aggressively metastatic breast cancer line MDA-MB-231. Notably, TTP-expressing cell lines replicate approximately 70% slower than non-transfected controls. Inhibition of cell proliferation did not result from apoptosis but rather by a delay at the G1/S checkpoint. TTP expression also significantly reduced the formation of cultured mammospheres based on both sphere-forming efficiency frequency and extreme limiting dilution analyses, indicating that this protein reduces stemness and non-adherent growth potential. Finally, cell motility was suppressed by TTP, demonstrated using wound healing assays. These findings are consistent with a tumor suppressor role for TTP. To identify potential mechanisms linking TTP to diminution of tumorigenic phenotypes, we surveyed the expression of genes encoding select pro-tumorigenic factors, including several known to encode TTP-targeted mRNAs. Expression of cyclin D1, cyclin E and c-Myc proteins were significantly reduced in TTP-expressing MDA-MB-231 cells, however, this was not mediated by accelerated mRNA decay. Interestingly, we observed that the mRNA-destabilizing function of TTP is abrogated by the constitutively active ERK signaling pathway in MDA-MB-231 cells, suggesting that TTP suppresses tumorigenic properties in this cell model independently of its canonical mRNA-destabilizing function. Consistent with this model, expressing a non-RNA-binding mutant form of TTP (C147R) robustly attenuated the same tumorigenic properties suppressed by the wild type protein. Together, these findings show that the mRNA-destabilizing activity of TTP is dispensable for its tumor suppressive properties in MDA-MB-231 cells, and by extension that TTP must limit diverse tumorigenic properties in these cells via an as yet uncharacterized non-canonical mechanism.

24. **An Oncogenic L1 Source Element Evades Somatic Repression and Initiates Human Colorectal Cancer**

Presenter: Emma Scott Mentor: Scott Devine
Department of Medicine

Transposable elements constitute ~45% of the human genome. The most common element is long interspersed element-1 (LINE-1, or L1), alone accounting for ~17% of the genome. While the majority of L1s are no longer active, a small subset of these autonomous retrotransposons remain capable of mobilizing themselves and pose an enormous mutagenic threat to the genome. In addition to L1 retrotransposition in the germline, which has been documented extensively, it has recently been established that L1s also are active in multiple types of human tumors and can change gene expression in cancer cells; thus, these elements may play an integral role in cancer development and progression. Here, we present an in-depth study of a model tumor that appears to have been initiated by L1 mutagenesis. We have identified a colorectal tumor with a somatic L1 insertion in an exon of the tumor suppressor APC; the majority of colorectal cancers are initiated by mutation and subsequent loss of function of both copies of APC, indicating that this L1 insertion was likely one of the first two genetic hits in a normal cell that caused progression to a dysblastic phenotype and, eventually, cancer. To study this in more detail we did whole genome sequencing and RNA-Seq of both the tumor and adjacent normal tissue. Using the MELT (Mobile Element Locator Tool) bioinformatic pipeline we identified and validated a total of 17 somatic L1 insertions in this tumor, and were able to track down the three non-reference (polymorphic) source elements responsible for generating most of these insertions. Two of these source elements, which together generated most of the somatic insertions in this tumor, are present in some individuals from the 1000 Genomes Project, and are population-specific. We also have evidence that the population-specific source element that caused the insertion into APC is expressed in the normal tissue. Collectively, these data support a model wherein activity of a population-specific oncogenic L1 in a normal colon cell initiated tumorigenesis.

25. **Concurrent Mutations in ATM and Common γ Chain Signal Transducers in Peripheral T cell Lymphoma**

Presenter: Haley Simpson Mentor: Arnob Banerjee
Department of Medicine

The 5-year overall survival for Peripheral T cell lymphoma (PTCL) is <40% due to poor response to current therapies and no targeted therapeutic options are yet available. As such, there is an urgent need to identify novel targets for PTCL therapy. Recent sequencing studies have identified recurrent activating mutations in the common gamma chain (γc)/JAK/STAT signaling pathway in PTCL, which may include potential cancer drivers. In line with this observation, a recent whole exome sequencing analysis by our lab identified 3 out of 12 PTCL cases with mutations in signal transducers of the γc signaling pathway: JAK3, IL2RG, and STAT5B. Two of these mutations, JAK3 M511I and STAT5B N642H, have been characterized as activating mutations in other hematologic malignancies. The third, γc K315E, was recently observed in one other case of PTCL but its function remains unknown. My research aims to improve our understanding of constitutively active γc signaling in PTCL by elucidating the role of γc K315E in PTCL oncogenesis and exploring STAT5 as a therapeutic target for PTCL treatment. We hypothesize that γc K315E is an oncogenic mutation that activates γc signaling in the absence cytokine stimulation. Aberrant activation of the γc, leading to cytokine independent growth and apoptotic resistance, has strong potential to be an oncogenic driver and potential therapeutic target in PTCL. The sequencing analysis also revealed a co-occurrence of γc signaling pathway mutations in cases also containing inactivating mutations in ATM (Ataxia Telangiectasia Mutated). Based on this observation, we explore potential synergism of STAT5 inhibition and PARP inhibition, to target ATM deficient cells, as a possible combination therapy for treatment of PTCL with this mutational landscape. Preliminary results support our hypothesis that STAT5 inhibition decreases PTCL cell proliferation and survival, alone and in combination with ATM deficiency. The overall goal of my research is to enhance understanding of the malignant processes that drive PTCL in order to identify new therapeutic targets and develop targeted therapeutic strategies to enhance treatment options for patients with this presently terminal diagnosis.

26. **Epigenetic Modulation of CD1d-mediated Antigen Presentation by B Cell Lymphomas**

Presenter: Irina Tiper Mentor: Tonya Webb
Department of Microbiology and Immunology

Histone deacetylases (HDACs) are a family of enzymes that regulate diverse cellular events such as gene expression, cell proliferation, and immune pathways through deacetylation of their protein targets. We hypothesize that tumors use epigenetic mechanisms to dysregulate CD1d-mediated antigen processing and presentation, which leads to a functional impairment in the ability of natural killer T (NKT) cells to recognize and destroy cancerous cells. We examined CD1d-mediated antigen presentation to NKT cells following treatment with HDAC inhibitors (HDACi). Consistent with previous studies, we found that preventing deacetylation by treatment with Trichostatin A, a pan-HDACi, enhanced both CD1d and MHC class II-mediated antigen presentation. Similarly, treatment of B cell lymphomas with Panobinostat resulted in increased CD1d-dependent NKT cell responses, due at least in part to a dose-dependent increase in CD1d-cell surface expression. In addition, treatment with HDCAi results in a decrease in STAT3 expression as well as IL-10 secretion by B cell lymphomas. Overall, our studies demonstrate the efficacy of HDACi in restoring anti-tumor responses to B cell lymphomas through both cell-intrinsic and extrinsic factors. Collectively, these results suggest that HDACi may work to enhance the immune response and increases the immunogenicity of the tumor itself.

27. **Identification and Characterization of Novel Immunosensitizing Therapeutics for Melanoma**

Presenter: Alexander Tsai Mentor: Eduardo Davila
Department of Microbiology and Immunology

Recent breakthroughs in the field of immunotherapy have revolutionized the treatment of patients with malignant melanoma. Specifically, immune checkpoint blockade of CTLA-4 and PD-1 have prolonged overall survival in a subset of melanoma patients. Despite dramatic and durable results in some cases, low overall response rates to these therapies leave the majority of melanoma patients without effective immunotherapeutic treatment options. In order to identify immunotherapies which might offer improved response rates with the potential to be rapidly translated to the clinic, we developed a high-throughput screen and tested over 2000 compounds, many of which are FDA-approved for various malignancies or other diseases. The screen revealed distinct families of multikinase inhibitors and DNA-damage repair inhibitors as novel melanoma immunosensitizing agents. We show that these two classes of drugs can reduce the expression of various immunosuppressive molecules (e.g. PD-L1, CD155) on the cell surface of a wide variety of genetically distinct human melanoma cell lines. Notably, the drugs also increased the expression of HLA and melanoma antigens in most cases. Importantly, preliminary data suggests that drug treatment sensitizes melanoma cells to T cell mediated cytotoxicity. Further preclinical studies are underway to verify drug targets, identify mechanisms of action, and establish in vivo anti-tumor activity.

28. **Preadipocyte Exosomes Promote Early Stage Breast Cancer Formation by Enhancing Cancer Stem Cell Renewal Signaling**

Presenter: Benjamin Wolfson Mentor: Qun Zhou
Department of Biochemistry and Molecular Biology

The tumor microenvironment plays a critical role in regulating breast cancer progression. Adipocytes differentiate from preadipocytes, which possess mesenchymal stem cell properties. Preadipocytes and adipocytes are essential components of the tumor microenvironment, and signaling between preadipocytes and breast cancer cells has been found to promote breast tumor formation and metastasis. Exosomes secreted from preadipocytes are also important components of the cancer stem cell niche. The primary goal of our studies is to examine how preadipocyte-derived exosomes can regulate early-stage breast cancer formation via regulating stem cell renewal. We use human and mouse preadipocytes and early-stage breast cancer cells (ductal carcinoma in situ, DCIS), as well as xenografts in immunodeficient mice to determine the role of preadipocyte secreted exosomes in DCIS stem cell behavior and tumor formation. By using protein arrays, we reveal CD81 and FLOT-1 as key exosomal protein markers and SOX2 and SOX9 as exosomal secretion of stem cell renewal regulators. We identify a key miR-140/SOX2/SOX9 axis that regulates differentiation, stemness, and migration in the tumor microenvironment. Next, we find that the natural antitumor compound shikonin can suppress preadipocyte signaling, inhibiting nearby DCIS cells. Through co-culture experiments we show that shikonin treated preadipocytes secrete exosomes containing high levels of miR-140 that can impact nearby DCIS cells through targeting SOX9 signaling. We demonstrate that preadipocyte-derived exosomes promote tumorigenesis in vivo, providing strong support for the importance of exosomal signaling in the tumor microenvironment. Finally, our data also show that targeting the tumor microenvironment may assist in blocking tumor formation.

Postdoctoral Fellows and Other Attendees:

29. **The Hippo Pathway in RUNX2-regulated Bone Metastasis in Breast Cancer: Transforming Growth Factor-β (TGF-β) -induced Modulation of TAZ Localization**

Presenter: Jessica Brusgard Mentor: Tony Passaniti
Department of Pathology

The Hippo pathway regulates organ size during development and its dysregulation leads to disease. Its role as a tumor suppressor pathway that modulates the transcriptional co-factors, YAP and TAZ, has become an active area of research. Disruption of cell:cell contacts results in the nuclear translocation of YAP and TAZ to enable their interaction with transcription factors, and the regulate cell proliferation and anti-apoptotic genes. These transcriptional regulators can interact with the master regulator of osteoblast differentiation and angiogenesis, RUNX2. RUNX2/TAZ promotes osteoblast differentiation, but their cooperative function in breast cancer is also of interest. Our lab and others have also shown that RUNX2 expression is correlated with breast cancer progression by promoting an osteomimetic phenotype allowing cancer cell homing to the bone and survival within the bone microenvironment. RUNX2 is overexpressed in early stage tumors; however, its role in these early stages has not yet been elucidated. Furthermore, TAZ has been suggested to promote an epithelial-mesenchymal transition in breast carcinoma. Since RUNX2 and TAZ cooperate to promote osteoblast differentiation, we have focused on examining the cooperation between RUNX2 and TAZ in promoting breast cancer progression through activation of a metastatic program. Treatment of an MCF7 cell line expressing inducible RUNX2 with TGF-β led to nuclear translocation of endogenous TAZ. This translocalization was preceded by disruption of adherens junctions through ectodomain shedding of E-Cadherin. RUNX2 positive cells released a soluble 80kDa oncogenic E-Cadherin (sE-Cad) fragment into the conditioned media. Further characterization of this novel molecular pathway will aid in the identification of new targets to prevent breast cancer metastasis to bone.

30. **X-ray Crystallographic Study of Small-molecules within the Persistent Binding Sites of S100B**

Presenter: Michael Cavalier Mentor: David Weber
Department of Biochemistry and Molecular Biology

S100B is not merely a prognostic indicator within Malignant Melanoma (MM), but it promotes the degradation of p53 through a calcium-dependent protein-protein interaction. It is also now known that small molecule inhibitors of S100B (known as SBiXs) and RNA interference of S100B expression restore both p53 protein levels and normal transcriptional activation/apoptosis activities in MM, which typically has wild-type p53. For these reasons, we aim to specifically inhibit the S100B-p53 interaction as a potential therapy for MM. Our efforts at probing the binding surface of S100B revealed three persistent binding sites (Sites 1, 2, and 3), and efforts to discover/synthesize SBiXs which simultaneously bind all three sites are underway. Using a combination of structural biology techniques (X-ray crystallography and NMR), in vitro binding, and cellular assays, the structure/function characterization of SBiXs binding Sites 1, 2 and 3 were investigated. The SBiXs, which exhibit dissociation constants ranging from 40 nM to 100 μM, are compared. Such inhibitors may also have therapeutic value as early stage lead compounds for treating other cancers such as astrocytoma, renal tumors, and some forms of leukemia that also have elevated S100B.

31. **Development of Biodegradable Fn14-targeted Nanoparticles for Controlled Drug Delivery for Invasive Brain Tumors**

Presenter: Nina Connolly Mentors: Graeme Woodworth, Anthony Kim, Jeff Winkles
Department of Neurosurgery

A major limitation associated with treatment of glioblastoma (GBM), the most common and deadly primary brain cancer, is delivery of therapeutics to invading tumor cells outside of the area that is safe for surgical removal. Recent advances in nanotechnology have allowed the incorporation of different therapeutic and targeting agents into nanoparticles offering the potential for improved detection, prevention, and treatment of various cancers. A promising way to target brain-invading GBM cells is via targeted therapeutics that bind to the cell surface receptor fibroblast growth-factor-inducible 14 (Fn14), which is specifically upregulated on the surface of invading GBM cells. In this study, we aim to develop a biodegradable nanoparticle platform that employs a dense, low-molecular weight PEG coating coupled with a Fn14-specific monoclonal antibody (mAb) in order to maximize brain tissue penetration and GBM cell targeting. We previously showed that PEG-coated model polystyrene (PS) nanoparticles conjugated to the Fn14 mAb named ITEM4 bind strongly and selectively to the Fn14 extracellular domain. We synthesized a variety of PS-based brain tissue penetrating PEG-coated nanoparticles and characterized the (i) specificity of nanoparticle binding to Fn14 and (ii) nonspecific binding to brain ECM components, using surface plasmon resonance (SPR) and multiple particle tracking (MPT) assays. In parallel, we are transferring these findings and methodology towards formulation of biodegradable drug-loaded nanoparticles with matched size, surface chemistries, and Fn14 binding affinities for controlled drug delivery into brain tumors. We are loading biodegradable nanoparticles, including poly(lactic-co-glycolic acid) (PLGA), polyglutamic acid (PGA), and polysebacic acid (PSA) polymer platforms, with chemotherapeutics (i.e. cisplatin and bis-chloroethylnitrosurea (BCNU)) to study the optimization of drug-loading with particle penetration and targeting. The equilibrium binding affinity (KD) of nanoparticles scaled nearly linear with the surface density of the ITEM4 molecules, indicating that the adhesiveness of nanoparticle formulations depends on the ITEM4 molecular presentation on the nanoparticle surface. PEG-coated Fn14-targeted nanoparticles of ~100 nm in diameter were able to rapidly penetrate brain tissue by MPT experiment in rat brain slices. In contrast, uncoated nanoparticles were immobilized in brain tissue. We have preliminary data that suggests we can develop biodegradable nanoparticles that provide sustained release of a wide range of drugs over several days. We have successfully encapsulated cisplatin and BCNU to the polymer backbone of PGA and PLGA containing a low-molecular weight PEG coating. Additional surface modifications have been made to enable Fn14 targeting by conjugating ITEM4 on the particle surface. Particles will undergo complete physicochemical characterization to optimize Fn14 targeting, nanoparticle movement, drug release kinetics, and in vivo efficacy. We have developed a nanoparticle platform that can diffuse and penetrate within brain tissue and selectively target remote experimental GBM tumors. Using this approach we can optimize therapeutics versions to improve drug efficacy while limiting many of the side effects and risks of free drug and non-targeted therapies.

32. **Semaphorin 4D Induces Myeloid Derived Suppressor Cells in Human Head and Neck Squamous Cell Carcinoma**

Presenter: Kyu Lee Han Mentor: Rania Younis
Department of Oncology and Diagnostic Sciences

Head and neck squamous cell carcinoma (HNSCC) remains a significant cause of cancer morbidity and mortality. It is marked by immune suppression, which is associated with increased level of suppressive immune cells. Semaphorin 4D (Sema4D) is a cellular mediator described to have several effects on various cellular and organ systems, including the immune system and is highly expressed by HNSCC. However, immunological function of Sema4D in the tumor microenvironment is yet to be elucidated. The purpose of this study was to determine the role of Sema4D produced by HNSCC in suppressing the immune system in the tumor microenvironment. First, we demonstrated that the conditioned media from HNSCC HN6 and HN13 cell lines, showed significant induction of Myeloid derived Suppressor cells (MDSCs) (CD33+, CD11b+, and HLA-DR-/low) from CD33+ cells separated from PBMCs of healthy donors by flow cytometry analysis and WST-1 proliferation assay. This increase in MDSCs corresponded with suppression of the autologous T cells.

To investigate the role of Sema4D produced by HNSCC in induction of the MDSCs, we used anti-Sema4D antibody, to block Sema4D in HN6 and HN13 conditioned media. This showed significant reduction in the MDSCs population (~50%). Furthermore, conditioned media from HN6 Sema4D-shRNA rescued the MDSCs-mediated T cell suppression. T cells proliferation and IFN-γ production recovered by 2 to 7 folds compared to controls co-cultured with myeloid cells. We observed decrease in MDSCs produced Arginase-1 as well as the immune suppressive cytokines, TGF-β, and IL-10 that showed 1.5 to 2.6 fold reduction in conditioned media from HN6 Sema4D-shRNA cells. Our data also showed increase of the effector T cells (CD4+ Tbet+ and CD8+ Tbet+), and decrease in immune tolerance T regulatory cells (CD4+ CD25+ FoxP3+) in different ratios of Myeloid to T cell co-culture grown in conditioned media of HN6 Sema4D-shRNA versus control shRNA. This study describes a novel immunosuppressive mechanism for Sema4D in HNSCC via induction of MDSCs, and features it as an immunotherapeutic target for future studies to enhance the anti-tumorigenic immune responses in HNSCC and other epithelial malignancies.

33. **Tubulin Acetylation in Breast Cancer**

Presenter: Lindsay Hessler Mentor: Stuart Martin
Department of Physiology

As a marker of stable microtubules, tubulin acetylation may be an important factor in breast cancer metastasis. HDAC inhibitors are novel cancer therapies that may unintentionally affect this pathway. Studying these medications in breast cancer cells may further support previous evidence that tubulin acetylation is associated with a metastatic phenotype. In the current study, breast cancer tumor cells were treated with a range of HDAC inhibitors. Western blotting, immunofluorescence, proliferation and reattachment were performed to analyze their effects. Results demonstrated that treatment of breast cancer tumor cells with HDAC inhibitors increases tubulin and histone acetylation without causing toxicity. Different classes of these medications caused different effects on histones and tubulin. The increase in tubulin acetylation (caused by HDAC inhibitors) results in a clear phenotypic change and possible functional alterations that may promote metastasis. Tubulin acetylation may be an important marker and therapeutic target in breast cancer. While HDAC inhibitors do increase levels of tubulin acetylation in vitro, further work is required to determine if this has any functional effect on tumor cells.

34. **SIRT6 Protein Deacetylase Interacts with MYH DNA Glycosylase, APE1 Endonuclease, and Rad9-Rad1-Hus1 Checkpoint Clamp to Promote Base Excision Repair**

Presenter: Bor-Jang Hwang Mentor: Lu-Chang A-Lien
Department of Biochemistry and Molecular Biology

SIRT6, a member of the NAD+-dependent histone/protein deacetylase family, regulates genomic stability, metabolism, and lifespan. Here, we show that SIRT6 interacts with and stimulates MYH glycosylase and APE1, two base excision repair enzymes involved in mutation avoidance from oxidative DNA damage. In addition SIRT6 interacts with the Rad9-Rad1-Hus1 (9-1-1) checkpoint clamp. The SIRT6-MYH interaction involves the inter-domain connector of MYH that also interacts with APE1 and 9-1-1. Mutagenesis studies indicate that SIRT6, APE1, and Hus1 bind overlapping but different sequence motifs on MYH. There is no competition of APE1, Hus1, or SIRT6 binding to MYH. Rather, one MYH partner enhances the association of the other two partners to MYH. Moreover, APE1 and Hus1 act together to stabilize the MYH/Sirt6 complex. Protein expression levels and auto mono-ADP-ribosylation of SIRT6 are dependent on MYH protein expression. This feedback regulation reveals a novel means to modulate SIRT6 activity. In addition, Myh foci induced by oxidative stress and Sirt6 depletion are frequently localized on mouse telomeres. Our findings indicate that SIRT6 forms a complex with MYH, APE1, and 9-1-1 to maintain genomic integrity particularly at telomeres.

35. **Novel Factors Regulating Cancer Cell's Survival**

Presenter: Raju Khatri Mentor: Michal Zalzman
Department of Biochemistry and Molecular Biology

Cancer cells are defined in part by their ability to evade the normal process of cellular aging, thereby becoming immortalized and exacting harm on their host. Therefore, cancer cell survival requires intact telomeres. Recently, ZSCAN4 has been shown to increase telomere length and genomic stability in mouse embryonic stem cells. A majority of the studies on ZSCAN4 have focused on the functional role of the mouse Zscan4c gene, primarily in pluripotent stem cells. However, little is known about the human ZSCAN4 gene and its implication in human cancer. Interestingly, our recent data demonstrates the expression of ZSCAN4 in human head and neck squamous cell carcinoma (HNSCC). The goal of the current research is to characterize the human ZSCAN4 protein in order to better understand its activity. To investigate a molecular mechanism by which ZSCAN4 contributes to HNSCC cell survival, we performed immunoprecipitation with ZSCAN4 antibody followed by mass spectroscopy. Furthermore, our preliminary data suggests that ZSCAN4 is degraded by a proteasome-dependent degradation pathway. Our research may establish the turnover rate of ZSCAN4 and establish the novel role of ZSCAN4 and its interacting factors as mediators of ZSCAN4 action in HNSCC cells.

36. **NME1 Mediates a Switch in Expression of Beta Integrin Subunits that Correlates with Prolonged Patient Survival**

Presenter: Katie Leonard Mentor: David Kaetzel
Department of Biochemistry and Molecular Biology

Expression of the metastasis suppressor NME1 in melanoma is associated with reduced cellular motility and invasion in vitro and metastasis in vivo, but the molecular mechanisms underlying this activity are not completely understood. Herein we report a novel mechanism through which NME1 modulates focal adhesion dynamics via regulation of integrin β1. Stable expression of NME1 significantly altered focal adhesion turnover at the cell periphery. Interestingly, over-expression of NME1 resulted in a switch in from predominantly fast recycling α4β1 integrins to slower recycling αvβ3 integrins. Contrary to its regulation of other cell surface receptors, the inhibition of integrin β1 by NME1 was found to occur at the transcriptional level rather than through dynamin-mediated endocytosis. This effect of NME1 required both its 3-5' exonuclease and nucleoside diphosphate kinase (NDPK) activities, which are also required for its metastasis suppressor activities in vivo. Furthermore, an inverse correlation was observed between NME1 and integrin β1 mRNA in a large cohort of primary melanoma biopsies. The inverse correlation of NME1 and integrin β1 RNA was also a strong predictor of prolonged distant disease-free and overall survival in patients with the basal-like subtype of breast carcinoma. Together, these data strongly suggest NME1 suppresses metastasis of human melanoma and some types of breast cancers by inhibiting integrin β1 expression, which reduces recycling of focal adhesions and suppresses cell motility.

37. **The Prostaglandin E Receptor EP4 is Upregulated on Breast Cancer Stem-like Cells and Regulates Sensitivity to Natural Killer Cells**

Presenter: Xinrong Ma Mentor: Amy Fulton
Department of Pathology

The cyclooxygenase-2 (COX-2) pathway is highly expressed in many breast tumors and contributes to poor outcomes. The COX-2 product prostaglandin E2 (PGE2) promotes tumor growth and metastasis by acting on the G-protein-coupled receptor EP4. Our goal is to understand the role that EP4 and COX-2 play in the survival of breast cancer cells with stem-like properties. We compared EP4 and COX-2 expression in mammosphere-forming and bulk populations derived from a panel of human and murine luminal and basal type tumor cells with different metastatic capacities. Expression of both EP4 and COX-2 were markedly increased in mammosphere-forming cells derived from basal and/or metastatic cells

relative to the bulk population, but neither COX-2 nor EP4 levels were elevated in mammospheres derived from luminal or non-metastatic cells. Breast cancer stem-like cells are more sensitive than the bulk population to inhibition with small molecule EP4 antagonists in vitro. Consistent with these data, tumor-initiating capacity in vivo is markedly inhibited by EP4 antagonists. PGE2 potently suppresses Natural Killer (NK) cell activities through the EP4 receptor and the administration of EP4 antagonists to tumor-bearing mice restores NK activities to normal levels. We asked if NK cells can recognize and lyse breast cancer stem cells and if EP4 plays a role in this process. We compared the lytic sensitivity of murine mammosphere-forming tumor cells versus the bulk population to cytotoxicity mediated by normal murine NK cells. In metastatic tumor cell lines 66.1 and 410.4, the breast cancer stem cell enriched population was less sensitive to NK-mediated lysis than the bulk population. Gene silencing of EP4 modestly increased the sensitivity of tumor target cells to NK killing which corresponded to increased expression of the NK activating ligand H60. We are delineating the mechanism by which EP4 and COX-2 are upregulated in cells with stem-like properties. Like EP4 and COX-2, STAT3 is upregulated in breast cancer stem cells. Inhibition of STAT3 reduces mammosphere-forming capacity. Our studies support a mechanism whereby COX-2/EP4 signaling induces STAT3 to support breast cancer stem cell survival by a feed-forward mechanism and that these cells may be relatively resistant to NK-mediated control. These studies support the continued examination of EP4 as a therapeutic target. In preclinical studies, EP4 antagonists protect NK cells from tumor-mediated immune suppression and thereby prevent tumor metastasis and inhibit cancer stem cells.

38. **DUOX2- the Key Player for Hyper-radiosensitivity in Gastric Cancer Cells with Low Dose Fractionation Radio Therapy**

Presenter: Palak Parekh Mentor: France Carrier
Department of Radiation Oncology

Radiotherapy is among the most conventional modality for solid tumors. Still, this modality can become challenging when it comes to managing highly disseminated gastrointestinal cancers due to toxicity to normal surrounding tissues. Low dose fractionated radiotherapy (LDFRT) was previously not considered since it was assumed that it would be ineffective for tumor removal. Recent evidence now indicate that LDFRT can produce hyper-radiosensitivity (HRS) and be combined with systemic chemotherapy. The aim of our study is to determine the efficacy of LDFRT in combination with modified regimen of docetaxel, cisplatin and 5'-fluorouracil (mDCF) and investigate their mechanism of action in gastric cancer cells. Our data indicate that three consecutive days of radiation with daily fraction of 0.15 Gy produced HRS in gastric cancer cells and potentiated the effect of chemotherapeutic drugs (mDCF). Colony survival assays showed that only 10% of gastric cancer cells survived when LDFRT was combined with mDCF while an almost ten times higher dose (1.35 Gy) of radiation was required to achieve the same results with radiation alone. RT2-PCR profiler array analysis showed marked upregulation of dual oxidase-2 (DUOX2), an enzyme of NADPH- oxidase family, in response to LDFRT combined with mDCF. This combined regimen did not induce genes involved in DNA repair. The functional significance of DUOX2 up-regulation was evident by a 3.5 fold increase in reactive oxygen species (ROS) in cells exposed to LDFRT and mDCF. Further, down regulation of DUOX2 expression increased resistance to radiation and chemoradiation. Taken together these data suggest that chemopotentiation by LDFRT in gastric cancer cells may be partly due to increased ROS production by DUOX2 without inducing the DNA repair machinery. These data thus provide a rationale for further explorations of potential clinical applications of LDFRT as a chemopotentiator for advanced and metastatic gastric cancers.

39. **Structural Evidence of Dimeric Napthoquinone Binding and Activation by Human NAD(P)H:Quinone Oxidoreductase 1**

Presenter: Swarna Pidugu Mentors: David Weber, Eric Toth
Department of Biochemistry and Molecular Biology

In contrast to the Warburg effect, some cancer cells rely on oxidative phosphorylation as main source of energy. Emadi et al, have recently showed that dimeric naphthoquinones (BiQ) undergo futile redox cycling that produce reactive oxygen species in yeast. This leads to cytotoxicity, disruption of mitochondrial membrane potential affecting its critical

functions like oxidative phosphorylation, lipid and pyrimidine synthesis and thereby leads to cell death. Moreover, the Emadi lab showed that BiQ cytotoxicity was specific to cancer cell lines that rely on oxidative phosphorylation such as DU145 cells but not PC-3 prostate cancer cell lines and similarly in MDA-453 cells but not MCF-7 cells. These results demonstrated that the phenomenon that they observed in yeast was replicated in mammalian cells. The target that facilitates BiQ toxicity is NAD(P)H: Quinone Oxidoreductase (NQO1) in mammals (mitochondrial external NAD(P)H dehydrogenase typeII, NDE1, in yeast). NQO1 is involved in detoxification of dietary quinones and activation of cancer chemotherapeutic quinones by two-electron reduction. NQO1 is overexpressed in several cancers making them more sensitive to BiQs. In this study, we present the crystal structure of human NQO1 complexed with a BiQ, E6a. This structure confirms the binding and activation of BiQ (E6a) by NQO1. This structural information will aid in future structure based optimization of BiQs for treatment of several cancers.

40. **The Role of the Metastasis-Suppressor NME1 in DNA Double-Strand Break Repair**

Presenter: Gemma Puts Mentor: David Kaetzel
Department of Biochemistry and Molecular Biology

NME1 is a potent suppressor of metastasis, and reduced NME1 expression has been associated with aggressive melanoma. NME1 protein possesses both nucleoside diphosphate kinase and 3'-5' exonuclease (3'-5' EXO) activities, both of which contribute to its metastasis suppressor activity. 3'-5' exonucleases are important for proofreading during DNA repair and DNA synthesis, suggesting a potential role for NME1 in maintenance of genomic stability in melanoma. Although impairment of DNA repair activity has been implicated in melanoma risk, its contribution to melanoma initiation and progression is not well understood. We have demonstrated that both enzymatic activities of NME1 are critical for repair of UV-induced DNA damage. More recently, we observed recruitment of NME1 to DNA double-strand breaks (DSBs) induced by the I-PpoI endonuclease, strongly suggesting participation of NME1 in DSB repair (DSBR). Moreover, repair of I-PpoI-induced DSBs in an NME-deficient melanoma line, WM793, was accelerated by forced NME1 expression. Induction of DSBs with gamma irradiation or bleomycin promoted rapid physical associations of NME1 with both ATM and NBS1, early sensors of DNA damage, further implicating NME1 in DSBR. Treatment with gamma irradiation also triggered a physical association between NME1 and XRCC4, an effector of the non-homologous end-joining (NHEJ) pathway of DSB repair. Intriguingly, forced expression of NME1 inhibited NHEJ-mediated repair of DSBs introduced in vitro. This suggests that NME1 may induce a switch from NHEJ to the homologous recombination pathway of DSBR, a model currently under examination in our laboratory. We have developed a lentiviral system capable of expressing I-PpoI in melanoma cell lines, which will enable verification and mapping of NME1 recruitment to those lesions. These data suggest direct participation of NME1 in DSBR, indicating a possible link between genomic instability and malignant progression in melanoma and potentially other cancers.

41. **Mir-300 Acts as a Tumor Suppressor in Ph+ Progenitors by Modulating the JAK2-SET/PP2A- B Catenin Interplay**

Presenter: Giovannino Silvestri Mentor: Danilo Perrotti
Department of Medicine

The persistence of tyrosine kinase inhibitor (TKI)-resistant malignant Philadelphia-positive (Ph+) hematopoietic stem cells (HSC) in chronic myelogenous leukemia (CML) TKI-treated patients in complete molecular remission, and the dismal prognosis of blast crisis CML indicate that the molecular mechanisms underlying its emergence, maintenance and progression are not totally dependent on the unrestrained kinase activity of BCR-ABL1 and might rely on other cell autonomous and/or microenvironmental signal capable of sustaining survival and self-renewal of the chronic phase and blast crisis Ph+ HSC compartment(s). We recently demonstrated that the Jak2/SET-PP2A/β-catenin pathway is essential for survival and self-renewal of quiescent TKI-resistant CD34+CD38- Ph+ HSC and that activation of such oncogenic signals requires the expression but not the activity of BCR-ABL1. Because microRNAs (miRNAs) are likely to control in a canonical and/or decoy manner the expression of different components of the Jak2 signalosome, this makes them an

attractive target for further understanding the mechanisms of leukemogenesis and, perhaps, for developing new alternative therapies that selectively eradicate quiescent leukemic HSCs. In silico analysis revealed that a specific miR subset shares multiple targets of the BCR-ABL1/Jak2/SET-PP2A signalosome. Nanostring Array analysis performed on primary bone marrow cells from normal individuals and chronic phase or blast crisis CML patients revealed that expression of some of these miRNA is altered in CML. For example, expression of miR-300 and miR-101, which are predicted to simultaneously modulate directly Jak2, hnRNP-A1 and β-catenin and, indirectly, other molecules of the BCR-ABL1/Jak2/SET-PP2A/β-catenin pathway, is significantly inhibited in chronic phase CML and further decreases in advanced CML samples. Additionally, miR-300 expression is several folds lower in dividing (div. 1) compared to quiescent (CFSEMAX) CD34+ CML cells. Lentiviral-transduction of miR-300 in human BCR-ABL+ cell lines resulted in marked downmodulation of JAK2, β-Catenin hnRNPA1 and SET and, as expected, in increased PP2A activity. Moreover, ectopic miR-300 expression decreased reduced clonogenic potential and proliferation, and increased susceptibility to TKI (e.g. Imatinib) induced apoptosis. Interestingly, it appears that forced BCR-ABL1 expression in TF-1 leukemic cells decreases miR-300, consistent with the reported activation in these cells of the Jak2-SET-PP2A-β-catenin pathway. Altogether our results suggest that miR-300 expression and, potentially, that of other deregulated non-coding RNAs might be dispensable or deleterious for the phenotype of Ph+ progenitors and/or indispensable for that of Ph+ HSCs. Thus, experiments in BCR-ABL1 cell lines as well as primary stem and progenitor cell fractions are currently ongoing to assess the role of this and other miRNAs in survival, self-renewal/proliferation of CML stem and progenitor cells.

42. **MSCs Prevent Imatinib-Induced Apoptosis and Induce Quiescence of BCR-ABL+ Cells Through Generation of PP2A-regulated Signals**

Presenter: Lorenzo Stramucci
Department of Medicine

Mentor: Danilo Perrotti

MicroRNA modulation of gene expression regulates diverse cellular processes, including erythropoiesis. Enforced expression of miR518c, which is aberrantly expressed in multiple cancers and preeclamptic placentas, conferred a growth advantage to human erythroleukemia TF1 cells in long-term growth competition assays. When TF1 cell cultures were transduced with a miR518c-expressing lentivirus marked with GFP (MOI < 1) the percentage of GFP+ cells increased progressively over time. MiR518c expression also conferred cytokine (GM-CSF) independence. MiR518c-transduced TF1 cells exhibited reduced apoptosis and proliferated in the absence of GM-CSF. When miR518c-transduced TF1 cells were stimulated with erythropoietin, a reduced fraction of CD34-CD235+CD71+ cells was observed compared to control cells, suggesting that miR518c inhibits early erythroid differentiation. The ability of miR518c to promote cytokine independence and inhibit differentiation may explain the association of miR518c with cancer. To determine whether miR518c can regulate primary hematopoietic stem-progenitor cell (HSPC) proliferation and/or differentiation, human CD34+ cells were transduced with miR518c vs. control lentiviruses. MiR518c-transduced HSPCs cultured in the presence of erythropoietin contained more CD34+ cells than control transduced cultures. In vitro colony-forming assays confirmed that miR518c-transduced HSPCs generated increased numbers of colony-forming cells. Taken together, our findings show that miR518c targets a gene(s) important in early erythroid proliferation/survival and differentiation. We are currently using gain- and loss-of-function approaches to determine which direct miR518c target(s) mediate the TF1 and primary HSPC phenotypes observed. Our goal is to take advantage of miR518c, as well as other microRNAs identified in our screen, to elucidate molecular pathways that regulate erythropoiesis.

43. **miRNAs Regulate Human Erythroid Progenitor Cell Growth and Differentiation**

Presenter: Brittney Taylor
Department of Pediatrics

Mentor: Tami Kingsbury

MicroRNA modulation of gene expression regulates diverse cellular processes, including erythropoiesis. Enforced expression of miR518c, which is aberrantly expressed in multiple cancers and preeclamptic placentas, conferred a growth advantage to human erythroleukemia TF1 cells in long-term growth competition assays. When TF1 cell cultures

were transduced with a miR518c-expressing lentivirus marked with GFP (MOI < 1) the percentage of GFP+ cells increased progressively over time. MiR518c expression also conferred cytokine (GM-CSF) independence. MiR518c-transduced TF1 cells exhibited reduced apoptosis and proliferated in the absence of GM-CSF. When miR518c-transduced TF1 cells were stimulated with erythropoietin, a reduced fraction of CD34-CD235+CD71+ cells was observed compared to control cells, suggesting that miR518c inhibits early erythroid differentiation. The ability of miR518c to promote cytokine independence and inhibit differentiation may explain the association of miR518c with cancer. To determine whether miR518c can regulate primary hematopoietic stem-progenitor cell (HSPC) proliferation and/or differentiation, human CD34+ cells were transduced with miR518c vs. control lentiviruses. MiR518c-transduced HSPCs cultured in the presence of erythropoietin contained more CD34+ cells than control transduced cultures. In vitro colony-forming assays confirmed that miR518c-transduced HSPCs generated increased numbers of colony-forming cells. Taken together, our findings show that miR518c targets a gene(s) important in early erythroid proliferation/survival and differentiation. We are currently using gain- and loss-of-function approaches to determine which direct miR518c target(s) mediate the TF1 and primary HSPC phenotypes observed. Our goal is to take advantage of miR518c, as well as other microRNAs identified in our screen, to elucidate molecular pathways that regulate erythropoiesis.

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