Dr. Haipeng Lui receives a new R56:
September 2014 2 years support to obtain preliminary data for an R01 resubmission.
Aim: To design molecular vaccines against Type I diabetes.


Older Patients with Myeloma Derive Similar Benefit from Autologous Transplantation.  Biol Blood Marrow Transplant. 2014 Jul 18.  PMID: 25046833

Abstract  Autologous hematopoietic cell transplantation (AHCT) for plasma cell myeloma is performed less often in people >70 years old than in people ≤70 years old. We analyzed 11,430 AHCT recipients for plasma cell myeloma prospectively reported to the Center for International Blood and Marrow Transplant Research between 2008 and 2011, representing the majority of US AHCT activity during this period. Survival (OS) was compared in 3 cohorts: ages 18 to 59 years (n = 5818), 60 to 69 years (n = 4666), and >70 years (n = 946). Median OS was not reached for any cohort. In multivariate analysis, increasing age was associated with mortality (P = .0006). Myeloma-specific mortality was similar among cohorts at 12%, indicating an age-related effect on nonmyeloma mortality. Analyses were performed in a representative subgroup comparing relapse rate, progression-free survival (PFS), and nonrelapse mortality (NRM). One-year NRM was 0% for age >70 years and 2% for other ages (P = not significant). The three-year relapse rate was 56% in age 18 to 59 years, 61% in age 60 to 69 years, and 63% age >70 (P = not significant). Three-year PFS was similar at 42% in age 18 to 59 years, 38% in age 60 to 69 years, and 33% in age >70 years (P = not significant). Postrelapse survival was significantly worse for the older cohort (P = .03). Older subjects selected for AHCT derived similar antitymela benefit without worse NRM, relapse rate, or PFS.

Fu Z, Abou-Samra AB, Zhang R
An explanation for recent discrepancies in levels of human circulating betatrophin.  Diabetologia. 2014 Oct;57(10):2232-4.  PMID: 25099942

LETTER  To the Editor:  In addition to hyperglycaemia and hyperinsulinaemia, type 2 diabetes mellitus is often associated with hypertriglyceridaemia. Recently, we and others have identified a novel circulating factor, referred to as lipasin, refeeding induced fat and liver (RIFL), angioptoin-like protein 8 (ANGPTL8), and betatrophin, which may have a dual role in mediating both triacylglycerol metabolism and glucose homeostasis. Lipasin mRNA levels in liver and fat, where it is predominantly expressed, are suppressed by fasting and highly induced by feeding and insulin resistance. Serum triacylglycerol levels are increased in lipasin overexpressing mice and decreased in lipasin knockout mice, and this likely occurs through a mechanism involving regulation of the activity of lipoprotein lipase (LPL) either directly or indi-recly by promoting ANGPTL3 cleavage. Indeed, the knockout mice exhibit higher LPL activity. The discovery of betatrophin as a potent and specific stimulator of pancreatic beta cell proliferation has drawn significant attention because it represents an intriguing therapeutic target for promoting beta cell regeneration in both type 1 and type 2 diabetes. As a logical next step to understanding the roles of betatrophin in human physiology and pathology, there is a growing interest in examining circulating levels of the protein in humans. Betatrophin levels have been found to be increased in type 1 and type 2 diabetes, and to be associated with atherogenic lipid profiles and BMI. However, there are notable discrepancies among results of these studies. For instance, levels of fasting betatrophin ranged from 0.3 ng/ml to 2.2 ng/ml in lean and on diabetic individuals; (2) were uncorrelated or positively correlated with BMI; and were either unaltered or increased in type 2 diabetes. We propose that all these results can be correct, but they reflect different betatrophin species, because different ELISA kits, although accurate and reliable, can generate different results because of betatrophin proteolytic regulation.
**Abstract**

Dietary energy balance modulates ovarian cancer progression and metastasis.

**OBJECTIVES:** The interaction of immune cells with adipocytes within the adipose tissues in obese persons with diabetes mellitus may play a role in insulin resistance. We examined in vitro whether nitric oxide (NO) and inducible nitric oxide synthase (iNOS) play a role in impaired insulin signalling in adipocytes exposed to activated macrophages.

**METHODS:** We used a co-culture system in which Raw264.7 macrophages were plated over differentiated, low passage 3T3-L1 cells (dif3T3) at a cell density ratio of 1:2. Inflammation was induced by a challenge with bacterial lipopolysaccharide.

**RESULTS:** Significantly (p<0.001) enhanced iNOS expression and NO synthesis was observed in activated co-cultures. In the co-cultures as compared with Raw264.7 cells alone, iNOS protein was induced up to 11-fold above background, and NO release was significantly (p<0.001) increased up to 2.8-fold. Co-culturing dif3T3 and Raw264.7 cells as compared to dif3T3 alone reduced insulin-induced Akt phosphorylation by 50% and AS160 phosphorylation by 42%. This was correlated with reduced glucose consumption when dif3T3 was exposed to 1,3-morpholinosyndonimine. Adiponectin, GLUT4 and AS160 mRNA were reduced by 4-fold, 5-fold and 2-fold, respectively, in co-cultures as compared to dif3T3 alone. On the contrary, GLUT1 mRNA levels were increased by 2-fold in co-cultures as compared to dif3T3. NG-monomethyl-L-arginine abolished NO production with modest reversal of Akt/AS160 phosphorylation.

**CONCLUSIONS:** This study demonstrated a potential association between iNOS/NO-mediated inflammation and insulin resistance.

---

**Zhang R, Abou-Samra AB**

**A dual role of lipasin (betatrophin) in lipid metabolism and glucose homeostasis: consensus and controversy.**

*Cardiovasc Diabetol.* 2014 Sep 13;13(1):133. PMID: 25212743

**Abstract**

Metabolic syndrome includes glucose intolerance and dyslipidemia, both of which are strong risk factors for developing diabetes and atherosclerotic cardiovascular diseases. Recently, multiple groups independently studied a previously uncharacterized gene, officially named C19orf80 (human) and Gm6484 (mouse), but more commonly known as RIFL, Angptl8, betatrophin and lipasin. Both exciting and conflicting results have been obtained, and significant controversy is ongoing. Accumulating evidence from genome wide association studies and mouse genetic studies convincingly shows that lipasin is involved in lipid regulation. However, the mechanism of action, the identity of transcription factors mediating its nutritional regulation, circulating levels, and relationship among lipasin, Angptl3 and Angptl4, remain elusive. Betatrophin represents a promising drug target for replenishing β-cell mass, but current results have not been conclusive regarding its potency and specificity. Here, we summarize the consensus and controversy regarding functions of lipasin/betatrophin based on currently available evidence.

**Five various collaborative works with Drs. Ali-Fehmi (TBM) Michele Cote (MT), Benjamin Rybicki (PSDR), Robert Morris (MT) and Wei Chen (MI) and Sandeep Mittal (MT) and Fazlul Sarkar (TBM) and Seema Sethi (MT) and S Bandyopadhyay (TBM)**


**Abstract**

OBJECTIVE: There are known disparities in endometrial cancer survival with black women who experience a greater risk of death compared with white women. The purpose of this investigation was to evaluate the role of comorbid conditions as modifiers of endometrial cancer survival by race. **STUDY DESIGN:** Two hundred seventy-one black women and 356 white women who had been diagnosed with endometrial cancer from 1990-2005 were identified from a large urban integrated health center. A retrospective chart review was conducted to gather information on comorbid conditions and other known demographic and clinical predictors of survival.

**RESULTS:** Black women experienced a higher hazard of death from any cause (hazard ratio [HR] 1.51; 95% confidence interval [CI], 1.22-1.87) and from endometrial cancer (HR, 2.42; 95% CI, 1.63-3.60). After adjustment for known clinical prognostic factors and comorbid conditions, the hazard of death for black women was elevated but no longer statistically significant for overall survival (HR, 1.22; 95% CI, 0.94-1.57), and the hazard of death from endometrial cancer remained significantly increased (HR, 2.27; 95% CI, 1.39-3.68). Both black and white women with a history of hypertension experienced a lower hazard of death from endometrial cancer (HR, 0.47; 95% CI, 0.23-0.98; and HR, 0.35; 95% CI, 0.19-0.67, respectively). **CONCLUSION:** The higher prevalence of comorbid conditions among black women does not explain fully the racial disparities that are seen in endometrial cancer survival. The association between hypertension and a lower hazard of death from endometrial cancer is intriguing, and further investigation into the underlying mechanism is needed.


**Dietary energy balance modulates ovarian cancer progression and metastasis.** *Oncotarget.* 2014 Aug 30;5(15):6063-75. PMID: 25026276

**Abstract**

A high energy balance, or caloric excess, accounts as a tumor promoting factor, while a negative energy balance via caloric restriction, has been shown to delay cancer progression. The effect of energy balance on ovarian cancer progression was investigated in an isogenic immunocompetent mouse model of epithelial ovarian cancer kept on a regimen of regular diet, high energy diet (HED) and caloric restricted diet (CRD), prior to inoculating the animals intraperitoneally with the mouse ovarian surface epithelial ID8 cancer cells. Tumor evaluation revealed that mice group on HED displayed the most extensive tumor formation with the highest tumor score at all organ sites (diaphragm, peritoneum, bowel, liver, kidney, spleen), accompanied with increased levels of insulin, leptin, insulin growth factor-1.
Tumor Biology and Microenvironment Program Publication Pipeline

(IGF-1), monocyte chemotactrant protein-1 (MCP-1), VEGF and interleukin 6 (IL-6). On the other hand, the mice group on CRD exhibited the least tumor burden associated with a significant reduction in levels of insulin, IGF-1, leptin, MCP-1, VEGF and IL-6.

Immunohistochemistry analysis of tumors from HED mice showed higher activation of Akt and mTOR with decreased adenosine monophosphate activated kinase (AMPK) and SIRT1 activation, while tumors from the CRD group exhibited the reverse profile. In conclusion, ovarian cancer growth and metastasis occurred more aggressively under HED conditions and was significantly curtailed under CRD. The suggested mechanism involves modulated secretion of growth factors, cytokines and altered regulation of AMPK and SIRT1 that converges on mTOR inhibition. While the role of a high energy state in ovarian cancer has not been confirmed in the literature, the current findings support investigating the potential impact of diet modulation as adjunct to other anticancer therapies and as possible individualized treatment strategy of epithelial ovarian cancer.


Abstract: Brain metastases from primary breast cancer are difficult to treat and associated with poor prognosis. Our understanding of the molecular basis for the development of such cancers is sparse. We hypothesized that the pro-metastatic microRNA-10b (miR-10b) plays a role in breast cancer brain metastasis. The study cohort comprised of twenty patients with breast cancer and brain metastasis as well as ten control patients (age, stage, and follow-up matched) with breast cancer without brain metastasis. All cases were microscopically reviewed to select tumor blocks with >50% tumor cells. RNA was extracted from formalin fixed paraffin embedded (FFPE) tumor tissue blocks. Expression of miR-10b was analyzed using qRT-PCR. The relevance of miR-10b expression was also tested using human breast cancer cell lines. An increased expression of miR-10b was noted in the primary breast cancer specimens of patients who subsequently developed brain metastasis, compared to those who did not. miR-10b also increased the invasive potential of breast cancer cells in vitro. Wilcoxon signed rank test revealed a statistically significant difference between the paired tumors from breast cancers and brain metastasis (p < 0.001). Increased expression of miR-10b appears to be associated with breast cancer brain metastasis. These findings are clinically relevant since miR-10b could serve as a prognostic and/or therapeutic target for anti-metastatic therapy. Identifying molecular signatures of primary breast cancers which have a propensity for brain metastasis is critical for designing novel therapies to counter the development of brain metastasis in patients diagnosed with breast cancer.


Abstract: Benign breast disease (BBD) is a very common condition, diagnosed in approximately half of all American women throughout their lifetime. While women with BBD are known to be at substantially increased risk of subsequent breast cancer, however, nothing is known about breast cancer characteristics that develop after a BBD diagnosis in African-American women. Here, we compared 109 breast cancers that developed in a population of African-American women with a history of BBD to 10,601 breast cancers that developed in a general population of African-American women whose cancers were recorded by the Metropolitan Detroit Cancer Surveillance System (MDCSS population). Demographic and clinical characteristics of the BBD population were compared to the MDCSS population, using chi-squared tests, Fisher’s exact tests, t-tests, and Wilcoxon tests where appropriate. Kaplan-Meier curves and Cox regression models were used to examine survival. Women in the BBD population were diagnosed with lower grade (p = 0.02), earlier stage cancers (p = 0.003) that were more likely to be hormone receptor-positive (p = 0.03) compared to the general metropolitan Detroit African-American population. In situ cancers were more common among women in the BBD cohort (36.7%) compared to the MDCSS population (22.1%, p < 0.001). Overall, women in the BBD population were less likely to die from breast cancer after 10 years of follow-up (p = 0.05), but this association was not seen when analyses were limited to invasive breast cancers. These results suggest that breast cancers occurring after a BBD diagnosis may have more favorable clinical parameters, but the majority of cancers are still invasive, with survival rates similar to the general African-American population.

Three works from R Arking. One in collaboration with LA Pile (MT)

Park JS, Pyo JH, Na HJ, Jeon HJ, Kim YS, Arking R, Yoo MA.

Abstract: Age-related changes in long-lived tissue-resident stem cells may be tightly linked to aging and age-related diseases such as cancer. Centrosomes play key roles in cell proliferation, differentiation and migration. Supernumerary centrosomes are known to be an early event in tumorigenesis and senescence. However, the age-related changes of centrosome duplication in tissue-resident stem cells in vivo remain unknown. Here, using anti-y-tubulin and anti-PH3, we analyzed mitotic intestinal stem cells with supernumerary centrosomes in the adult Drosophila midgut, which may be a versatile model system for stem cell biology. The results showed increased centrosome amplification in intestinal stem cells of aged and oxidatively stressed Drosophila midgut. Increased centrosome amplification was detected by overexpression of PVR, EGFR, and AKT in intestinal stem cells/enteroblasts, known to mimic age-related changes including hyperproliferation of intestinal stem cells and hyperplasia in the midgut. Our data show the first direct evidence for the age-related increase of centrosome amplification in intestinal stem cells and suggest that the Drosophila midgut is an excellent model for studying molecular mechanisms underlying centrosome amplification in aging adult stem cells in vivo.
Barnes VL, Bhat A, Unnikrishnan A, Heydari AR, Arking R, Pile LA.


Abstract Coordinate control of gene activity is critical for fitness and longevity of an organism. The SIN3 histone deacetylase (HDAC) complex functions as a transcriptional repressor of many genes. SIN3-regulated genes include those that encode proteins affecting multiple aspects of mitochondrial function, such as energy production and stress responsiveness, important for health maintenance. Here we used Drosophila melanogaster as a model organism to examine the role of SIN3 in the regulation of fitness and longevity. Adult flies with RNA interference (RNAi) induced knockdown expression of Sin3A have reduced climbing ability; an activity that likely requires fully functional mitochondria. Additionally, compared to wild type, adult Sin3A knockdown flies were more sensitive to oxidative stress. Interestingly, media supplementation with the antioxidant glutathione largely restored fly tolerance to oxidative stress. Although Sin3A knockdown flies exhibited decreased longevity compared to wild type, no significant changes in expression of many well-categorized aging genes were observed. We found, however, that Sin3A knockdown corresponded to a significant reduction in expression of genes encoding proteins involved in the de novo synthesis of glutathione. Taken together, the data support a model whereby SIN3 regulates a gene expression program required for proper mitochondrial function and effective stress response during adulthood.


Abstract Multiple studies characterizing the human ageing phenotype have been conducted for decades. However, there is no centralized resource in which data on multiple age-related changes are collated. Currently, researchers must consult several sources, including primary publications, in order to obtain age-related data at various levels. To address this and facilitate integrative, system-level studies of ageing we developed the Digital Ageing Atlas (DAA). The DAA is a one-stop collection of human age-related data covering different biological levels (molecular, cellular, physiological, psychological and pathological) that is freely available online (http://ageing-map.org/). Each of the >3000 age-related changes is associated with a specific tissue and has its own page displaying a variety of information, including at least one reference. Age-related changes can also be linked to each other in hierarchical trees to represent different types of relationships. In addition, we developed an intuitive and user-friendly interface that allows searching, browsing and retrieving information in an integrated and interactive fashion. Overall, the DAA offers a new approach to systemizing ageing resources, providing a manually-curated and readily accessible source of age-related changes.


Abstract Subpopulations of cancer stem cells (CSCs) or cancer stem-like cells (CSLCs) have been identified from most tumors, including pancreatic cancer (PC), and the existence of these cells is clinically relevant. Emerging evidence suggests that CSLCs participate in cell growth/proliferation, migration/invasion, metastasis, and chemo-radiotherapy resistance, ultimately contributing to poor clinical outcome. However, the pathogenesis and biological significance of CSLCs in PC has not been well characterized. In the present study, we found that isolated triple-marker-positive (CD44(+)/CD133(+)/EpCAM(+)) cells of human PC MiaPaCa-2 and L3.6pl cells behave as CSLCs. These CSLCs exhibit aggressive behavior, such as increased cell growth, migration, clonogenicity, and self-renewal capacity. The mRNA expression profiling analysis showed that CSLCs (CD44(+)/CD133(+)/EpCAM(+)) exhibit differential expression of more than 1,600 mRNAs, including FoxQ1, compared with the triple-marker-negative (CD44(-)/CD133(-)/EpCAM(-)) cells. The knockdown of FoxQ1 by its siRNA in CSLCs resulted in the inhibition of aggressive behavior, consistent with the inhibition of EpCAM and Snail expression. Mouse xenograft tumor studies showed that CSLCs have a 100-fold higher potential for tumor formation and rapid tumor growth, consistent with overexpression of CSC-associated markers/mediators, including FoxQ1, compared with its parental MiaPaCa-2 cells. The inhibition of FoxQ1 attenuated tumor formation and growth, and expression of CSC markers in the xenograft tumor derived from CSLCs of MiaPaCa-2 cells. These data clearly suggest the role of differentially expressed genes in the regulation of CSLC characteristics, further suggesting that targeting some of these genes could be important for the development of novel therapies for achieving better treatment outcome of PC.


Abstract SUMOylation is an essential posttranslational modification and regulates many cellular processes. Dysregulation of SUMOylation plays a critical role in metastasis, yet how its perturbation affects this lethal process of cancer is not well understood. We found that SUMO-2/3 modification is greatly up-regulated in metastatic breast cancer cells compared with nonmetastatic control cells. To identify proteins differentially modified by SUMO-2/3 between metastatic and nonmetastatic cells, we established a method in which endogenous SUMO-2/3 conjugates are labeled by stable isotope labeling by amino acids in cell culture (SILAC), immunopurified by SUMO-2/3 monoclonal antibodies and epitope-antibody elution, and analyzed by quantitative mass spectrometry. We identified 66 putative SUMO-2/3-conjugated proteins, of which 15 proteins show a significant increase/decrease in SUMO-2/3 modification in metastatic cells. Targets with altered SUMOylation are involved in cell cycle, migration, inflammation, glycolysis, gene expression, and SUMO/ubiquitin pathways, suggesting that perturbations of SUMO-2/3 modification might contribute to metastasis by affecting these processes. Consistent with this, up-regulation of PML SUMO-2/3 modification corresponds to an increased number of PML nuclear bodies (PML-NBs) in metastatic cells, whereas up-regulation of global SUMO-2/3 modification promotes 3D cell migration. Our findings provide a foundation for further investigating the effects of SUMOylation on breast cancer progression and metastasis.
Approximately June 25 2014


**Abstract** Purpose: Biotinidase deficiency, if untreated, usually results in neurological and cutaneous symptoms. Biotin supplementation markedly improves and likely prevents symptoms in those treated early. All states in the United States and many countries perform newborn screening for biotinidase deficiency. However, there are few studies about the outcomes of the individuals identified by newborn screening. Methods: We report the outcomes of 142 children with biotinidase deficiency identified by newborn screening in Michigan over a 25-year period and followed in our clinic; 22 had profound deficiency and 120 had partial deficiency. Results: Individuals with profound biotinidase and partial deficiency identified by newborn screening were started on biotin therapy soon after birth. With good compliance, these children appeared to have normal physical and cognitive development. Although some children exhibited mild clinical problems, these are unlikely attributable to the disorder. Biotin therapy appears to prevent the development of neurological and cutaneous problems in our population. Conclusion: Individuals with biotinidase deficiency ascertained by newborn screening and treated since birth appeared to exhibit normal physical and cognitive development. If an individual does develop symptoms, after compliance and dosage issues are excluded, then other causes must be considered.


**Abstract** Tissue inhibitor of metalloproteinase-1 (TIMP-1) regulates intracellular signaling networks for inhibition of apoptosis. Tetraspanin (CD63), a cell surface binding partner for TIMP-1, was previously shown to regulate integrin-mediated survival pathways in the human breast epithelial cell line MCF10A. In the current study, we show that TIMP-1 expression induces phenotypic changes in cell morphology, cell adhesion, cytoskeletal remodeling, and motility, indicative of an epithelial-mesenchymal transition (EMT). This is evidenced by loss of the epithelial cell adhesion molecule E-cadherin with an increase in the mesenchymal markers vimentin, N-cadherin, and fibronectin. Signaling through TIMP-1, but not TIMP-2, induces the expression of TWIST1, an important EMT transcription factor known to suppress E-cadherin transcription, in a CD63-dependent manner. RNAi-mediated knockdown of TWIST1 rescued E-cadherin expression in TIMP-1-overexpressing cells, demonstrating a functional significance of TWIST1 in TIMP-1-mediated EMT. Furthermore, analysis of TIMP-1 structural mutants reveals that TIMP-1 interactions with CD63 that activate cell survival signaling and EMT do not require the matrix metalloproteinase (MMP)-inhibitory domain of TIMP-1. Taken together, these data demonstrate that TIMP-1 binding to CD63 activates intracellular signal transduction pathways, resulting in EMT-like changes in breast epithelial cells, independent of its MMP-inhibitory function. **IMPLICATIONS:** TIMP-1’s function as an endogenous inhibitor of MMP or as a “cytokine-like” signaling molecule may be a critical determinant for tumor cell behavior.

**Two new papers for Dr. Granneman et al**

Mottillo EP, Balasubramanian P, Lee YH, Weng C, Kershaw EE, **Granneman JG**

**Coupling of lipolysis and de novo lipogenesis in brown, beige, and white adipose tissues during chronic β3-adrenergic receptor activation.** J Lipid Res. 2014 Sep 5. [Epub ahead of print]. PMID: 25193997

**Abstract** Chronic activation of β3-adrenergic receptors (β3-ARs) expands the catabolic activity of both brown adipose tissue (BAT) and white adipose tissue (WAT) by engaging UCP1-dependent and UCP1 independent processes. The present work examined de novo lipogenesis (DNL) and triglyceride (TG)/glycerol dynamics in classic brown, subcutaneous 'beige', and classic WAT during sustained β3-AR activation by CL 316,243 (CL), and also addressed the contribution of TG hydrolysis to these dynamics. CL treatment for 7 days dramatically increased DNL and TG turnover similarly in all adipose depots, despite great differences in UCP1 abundance. Increased lipid turnover was accompanied by the simultaneous upregulation of genes involved in both fatty acid synthesis, glycerol metabolism, and fatty acid oxidation. Inducible, adipocyte-specific deletion of adipose triglyceride lipase (ATGL), the rate limiting enzyme for lipolysis, demonstrates that TG hydrolysis is required for CL-induced increases in DNL, TG turnover, and mitochondrial electron transport in all depots. Interestingly, the effect of ATGL deletion on induction of specific genes involved in fatty acid oxidation and synthesis varied among fat depots. Overall, these studies indicate that fatty acid synthesis and oxidation are tightly coupled in adipose tissues during chronic adrenergic activation, and this effect critically depends on the activity of adipocyte ATGL.

Contreras GA, Lee YH, Mottillo EP, **Granneman JG**


**Abstract** Brown adipocytes (BA) generate heat in response to sympathetic activation and are the main site of non-shivering thermogenesis in mammals. Although most BA are located in classic brown adipose tissue depots, BA are also abundant in the inguinal white adipose tissue (iWAT) prior to weaning. The number of BA is correlated with the density of sympathetic innervation in iWAT; however, the role of continuous sympathetic tone in the establishment and maintenance of BA in iWAT has not been investigated. BA marker expression in iWAT was abundant in weaning mice, but was greatly reduced by 8 weeks of age. Nonetheless, BA phenotype could be rapidly reinstated by acute β3 adrenergic stimulation with CL 316,243 (CL). Genetic tagging of adipocytes with adiponectin-CreER(T2) demonstrated CL reinstates UCP1 expression in adipocytes that were present prior to weaning. Chronic surgical denervation dramatically reduced the ability of CL to induce the expression of UCP1 and other BA markers in the tissue as a whole, and this loss of responsiveness was prevented by concurrent treatment with CL. These results indicate that ongoing sympathetic activity is critical to preserve the ability of iWAT fat cells to express BA phenotypes upon adrenergic stimulation.
Jacques SM, Kupsky WJ, Qureshi F

Abstract
Abstract Objective: With advances in therapy, more neonates with severe congenital anomalies are surviving, albeit some with neurologic disorders, possibly related to antenatal low brain blood flow. This autopsy series reports antenatal brain injury in neonates expiring due to severe anomalies, and provides correlation with umbilical cord blood gas and acid-base analysis. Methods: We identified autopsies of third trimester neonates expiring shortly following delivery due to severe anomalies or malformations. Brain injury classified as “older” included periventricular leukomalacia, gliosis and karyorrhectic neurons, and “recent” included red neurons and reactive gli changes. Results: We identified 22 cases (nine term, 13 preterm). 16 (73%) had brain injury, including 11 with older injury. Cord arterial blood was analyzed in 17, and six had pH <7 or base deficit >12 mmol/L. Four out of 5 (80%) neonates with neuronal necrosis compared to two out of 12 (17%) without had a pH <7 or base deficit >12 mmol/L (p = 0.03). Five out of nine (56%) neonates with white matter injury compared to one out of 8 (13%) without had pH <7 or base deficit >12 mmol/L (p = NS). Conclusions: Antenatal brain injury is frequent in neonates with severe congenital anomalies. Severely abnormal cord blood analysis results correlate significantly with neuronal necrosis and show a trend toward white matter injury; however, the absence of these abnormal results does not preclude the presence of brain injury.

Laukka JJ, Makki MI, Lafleur T, Stanley J, Kamholz J, Garbney JY

Abstract
Pelizaeus-Merzbacher disease (PMD) is an X-linked disorder of the central nervous system (CNS) caused by a wide variety of mutations affecting proteolipid protein 1 (PLP1). We assessed the effects of PLP1 mutations on water diffusion in CNS white matter by using diffusion tensor imaging. Twelve patients with different PLP1 point mutations encompassing a range of clinical phenotypes were analyzed, and the results were compared with a group of 12 age-matched controls. The parallel (λ//), perpendicular (λ⊥), and apparent diffusion coefficients (ADC) and fractional anisotropy were measured in both limbs of the internal capsule, the genu and splenium of corpus callosum, the base of the pons, and the cerebral peduncles. The mean ADC and λ⊥ in the PMD patient group were both significantly increased in all selected structures, except for the base of the pons, compared with controls. PMD patients with the most severe disease, however, had a significant increase of both λ// and λ⊥. In contrast, more mildly affected patients had much smaller changes in λ// and λ⊥. These data suggest that myelin, the structure responsible in part for the λ⊥ barrier, is the major site of disease pathogenesis in this heterogeneous group of patients. Axons, in contrast, the structures mainly responsible for λ//, are much less affected, except within the subgroup of patients with the most severe disease. Clinical disability in patients with PLP1 point mutation is thus likely determined by the extent of pathological involvement of both myelin and axons, with alterations of both structures causing the most severe disease.

Mishra M, Zhong Q, Kowluru RA.

Abstract
Diabetes increases oxidative stress in the retina and decreases the levels of the intracellular antioxidant glutathione (GSH). The transcriptional factor Nrf2 regulates the expression of Gclc, the enzyme important in the biosynthesis of GSH, and in diabetes the binding of Nrf2 to the antioxidant response element region 4 (ARE4) is decreased. Our aim was to investigate the role of epigenetic modifications in the decreased Nrf2 binding at Gclc-ARE4 in the development of diabetic retinopathy and in the metabolic memory associated with its continued progression. The effect of hyperglycemia on H3K4 methylation in Nrf2 binding at Gclc-ARE4 was investigated by chromatin immunoprecipitation in the rat retina and was confirmed in retinal endothelial cells in which histone demethylase (LSD1) was manipulated. The role of histone methylation at Gclc-ARE4 in the metabolic memory was examined in rats maintained under poor control for 3 months followed by good control (GC) for 3 months. Although H3K4me2 at Gclc-ARE4 was increased in diabetes, H3K4me3 and H3K4me1 were decreased. LSD1 siRNA abrogated the glucose-induced decrease in H3K4me1 at Gclc-ARE4 and ameliorated decreases in Nrf2 binding at Gclc-ARE4 and Gclc transcripts. Reestablishment of GC failed to provide any benefits to histone methylation, and Nrf2 binding activity remained compromised. Thus, in diabetic retinopathy, histone methylation at Gclc-ARE4 plays an important role in regulating the Nrf2-Gclc-GSH cascade. Targeting histone methylation could help inhibit/slow down this blinding disease.

Bao J, Wang S, Gunther LK, Kitajiri SI, Li C, Sakamoto T

The actin-bundling protein TRIOBP-4 and -5 promotes the motility of pancreatic cancer cells. Abstract TRIOBP isoforms 4 and 5 (TRIOBP-4/5) are an actin-bundling protein associated with hearing loss. Here, we showed that TRIOBP-4/5 was up-regulated in human pancreatic carcinoma cells. Knockdown of TRIOBP-4/5 led to a loss of filopodia and a decrease in cell motility. Confocal microscopy showed that re-expression of GFP-TRIOBP-4 or -5 restored the filopodial formation in TRIOBP-4/5-deficient PANC-1 cells. Finally, TRIOBP-4/5 was shown to be overexpressed in human pancreatic cancer tissues. These results demonstrate a novel role of TRIOBP-4/5 that promotes the motility of pancreatic cancer cells via regulating actin cytoskeleton reorganization in the filopodia of the cells.
Yano H, Thakur A, Tomaszewski EN, Choi M, Deol A, Lum LG


**Abstract**

**BACKGROUND:** Ipiilimumab is an antagonistic monoclonal antibody against cytotoxic T-lymphocyte antigen-4 (CTLA-4) that enhances antitumor immunity by inhibiting immunosuppressive activity of regulatory T cells (Treg). In this study, we investigated whether inhibiting Treg activity with ipilimumab during ex vivo T cell expansion could augment anti-CD3-driven T cell proliferation and enhance bispecific antibody (BiAb)-redirected antitumor cytotoxicity of activated T cells (ATC).

**METHODS:** PBMC from healthy individuals were stimulated with anti-CD3 monoclonal antibody with or without ipilimumab and expanded for 10-14 days. ATC were harvested and armed with anti-CD3 x anti-EGFR BiAb (EGFRBi) or anti-CD3 x anti-CD20 BiAb (CD20Bi) to test for redirected cytotoxicity against COLO356/FG pancreatic cancer cell line or Burkitt’s lymphoma cell line (Daudi).

**RESULTS:** In PBMC from healthy individuals, the addition of ipilimumab at the initiation of culture significantly enhanced T cell proliferation (p = 0.0029). ATC grown in the presence of ipilimumab showed significantly increased mean tumor-specific cytotoxicity at effector:target (E:T) ratio of 25:1 directed at COLO356/FG and Daudi by 37.71% (p < 0.0004) and 27.5% (p < 0.0004), respectively, and increased the secretion of chemokines (CCL2, CCL3, CCL4, CCL5, CXCL9, and granulocyte-macrophage colony stimulating factor (GM-CSF)) and cytokines (IFN-γ, IL-2R, IL-12, and IL-13), while reducing IL-10 secretion.

**CONCLUSIONS:** Expansion of ATC in the presence of ipilimumab significantly improves not only the T cell proliferation but it also enhances cytokine secretion and the specific cytotoxicity of T cells armed with bispecific antibodies.

Liu X, Gao N, Dong C, Zhou L, Mi QS, Standiford TJ, Yu FS


**Abstract**

We previously showed that topical flagellin induces profound mucosal innate protection in the cornea against microbial infection, involving multiple genes and cell types. In this study, we used a Candida albicans (CA)-C57BL/6 mouse keratitis model to delineate the contribution of CXCL10- and CXCR3-expressing cells in flagellin-induced protection. Flagellin pretreatment markedly enhanced CXCL10 expression at 6 h post CA infection (hpi), but significantly dampened CXCL10 expression at 24 hpi. At the cellular level, CXCL10 was expressed in the epithelia at 6 hpi in flagellin-pretreated corneas, and concentrated at lesion sites 24 hpi. CXCR3-expressing cells were detected in great numbers at 24 hpi, organized within clusters at the lesion sites in CA-infected corneas. CXCL10 or CXCR3 neutralization increased keratitis severity and dampened flagellin-induced protection. CXCR3-positive cells were identified as NK cells, the depletion of which resulted in severe CA keratitis. Contributions from NK T-cells were excluded by finding no change in flagellin-induced protection in Rag1 KO mice. Recombinant CXCL10 inhibited CA growth in vitro and accelerated fungal clearance and inflammation resolution in vivo. Taken together, our data indicate that epithelium-expressed CXCL10 plays a critical role in fungal clearance and that CXCR3-expressing NK cells contribute to CA eradication in mouse corneas.

Funasaka T, Raz A, Nangia-Makker P.


**Abstract**

Galectin-3 is a member of the family of β-galactoside-binding lectins characterized by evolutionarily conserved sequences defined by structural similarities in their carbohydrate-recognition domains. Galectin-3 is a unique, chimeric protein consisting of three distinct structural motifs: (i) a short NH2 terminal domain containing a serine phosphorylation site; (ii) a repetitive proline-rich collagen-like sequence cleavable by matrix metalloproteinases; and (iii) a globular COOH-terminal domain containing a carbohydrate-binding motif and an NWGR anti-death motif. It is ubiquitously expressed and has diverse biological functions depending on its subcellular localization. Galectin-3 is mainly found in the cytoplasm, also seen in the nucleus and can be secreted by non-classical, secretory pathways. In general, secreted galectin-3 mediates cell migration, cell adhesion and cell-cell interactions through the binding with high affinity to galectose-containing glycoproteins on the cell surface. Cytoplasmic galectin-3 exhibits anti-apoptotic activity and regulates several signal transduction pathways, whereas nuclear galectin-3 has been associated with pre-mRNA splicing and gene expression. Its unique chimeric structure enables it to interact with a plethora of ligands and modulate diverse functions such as cell growth, adhesion, migration, invasion, angiogenesis, immune function, apoptosis and endocytosis emphasizing its significance in the process of tumor progression. In this review, we have focused on the role of galectin-3 in tumor metastasis with special emphasis on angiogenesis.

Michel AK, Nangia-Makker P, Raz A, Cloninger MJ.


**Abstract**

By using lactose-functionalized poly(amidoamine) dendrimers as a tunable multivalent platform, we studied cancer cell aggregation in three different cell lines (A549, DU-145, and HT-1080) with galectin-3. We found that small lactose-functionalized G(2)-dendrimer 1 inhibited galectin-3-induced aggregation of the cancer cells. In contrast, dendrimer 4 (a larger, generation 6 dendrimer with 100 carbohydrate end groups) caused cancer cells to aggregate through a galectin-3 pathway. This study indicates that inhibition of cellular aggregation occurred because 1 provided competitive binding sites for galectin-3 (compared to its putative cancer cell ligand, TF-antigen on MUC1). Dendrimer 4, in contrast, provided an excess of ligands for galectin-3 binding; this caused crosslinking and aggregation of cells to be increased.

Goodman CK, Wolfenden ML, Nangia-Makker P, Michel AK, Raz A, Cloninger MJ.


**Abstract**

Galectin-3 mediates cell surface glycoprotein clustering, cross linking, and lattice formation. In cancer biology, galectin-3 has been reported to play a role in aggregation processes that lead to tumor embolization and survival. Here, we show that lactose-functionalized dendrimers interact with galectin-3 in a multivalent fashion to form aggregates. The glycodendrimer-galectin...
aggregates were characterized by dynamic light scattering and fluorescence microscopy methodologies and were found to be discrete particles that increased in size as the dendrimer generation was increased. These results show that nucleated aggregation of galectin-3 can be regulated by the nucleating polymer and provide insights that improve the general understanding of the binding and function of sugar-binding proteins.


**Omega-3 fatty acid is a potential preventive agent for recurrent colon cancer.** Cancer Prev Res (Phila). 2014 Sep 5. [Epub ahead of print] PMID: 25193342

**Abstract** Increasing evidence supports the contention that many malignancies, including sporadic colorectal cancer (CRC), are driven by the self-renewing, chemotherapy-resistant cancer stem/stem-like cells (CSCs/CSLCs) underscoring the need for improved preventive and therapeutic strategies targeting CSCs/CSCs. Omega-3 polyunsaturated fatty acids (ω-3 PUFA), have been reported to inhibit the growth of primary tumors, but their potential as a preventive agent for recurring cancers is unexplored. The primary objectives of this investigation are to examine whether eicosapentaenoic acid (EPA; one of the ω-3 PUFA) synergizes with FuOx (5-FU+Oxaliplatin), the backbone of colon cancer chemotherapy, and (b) whether EPA by itself or in combination with conventional chemotherapy prevents the recurrence of colon cancer via eliminating/suppressing CSCs/CSCs. FuOx-resistant (chemo-resistant; CR) colon cancer cells, highly enriched in CSCs, were utilized for this study. While EPA alone was effective, combination of EPA and FuOx was more potent in (a) inhibiting cell growth, colonosphere formation and sphere-forming frequency, (b) increasing sphere disintegration, (c) suppressing the growth of SCID mice xenografts of CR colon cancer cells, and (d) decreasing pro-inflammatory metabolites in mice. Additionally, EPA + FuOx caused a reduction in CSC/CSC population. The growth reduction by this regimen is the result of increased apoptosis as evidenced by PARP cleavage. Furthermore, increased pPTEN, decreased pAkt, normalization of β-catenin expression, localization and transcriptional activity by EPA suggests a role for PTEN/Akt axis and Wnt signaling in regulating this process. Our data suggest that EPA by itself or in combination with FuOx could be an effective preventive strategy for recurring CRC.

Wang NY, Patras KA, Seo HS, Cavaco CK, Rösler B, Neely MN, Sullam PM, Doran KS.


**Abstract** Group B streptococcus (GBS) can cause severe disease in susceptible hosts, including newborns, pregnant women, and the elderly. GBS serine-rich repeat (Srr) surface glycopolypeptides are important adhesins/invasins in multiple host tissues, including the vagina. However, exact molecular mechanisms contributing to their importance in colonization are unknown. We have recently determined that Srr proteins contain a fibrinogen-binding region (BR) and hypothesize that Srr-mediated fibrinogen binding may contribute to GBS cervicovaginal colonization. In this study, we observed that fibrinogen enhanced wild-type GBS attachment to cervical and vaginal epithelium, and that this was dependent on Srr1. Moreover, purified Srr1-BR peptide bound directly to host cells, and peptide administration in vivo reduced GBS recovery from the vaginal tract. Furthermore, a GBS mutant strain lacking only the Srr1 “latching” domain exhibited decreased adherence in vitro and decreased persistence in a mouse model of GBS vaginal colonization, suggesting the importance of Srr-fibrinogen interactions in the female reproductive tract.

...from our Program members in Canada

Fidalgo da Silva E, Botsford S, Porter LA.


**Abstract** Progression through G2 phase of the cell cycle is a technically difficult area of cell biology to study due to the lack of physical markers specific to this phase. The FUCCI system uses the biology of the cell cycle to drive fluorescence in select phases of the cell cycle. Similarly, a commercially available system has used a fluorescent analog of the Cyclin B1 protein to visualize cells from late S to metaphase-anaphase transition. We have modified these systems to use the promoter and destruction box elements of Cyclin B1 to drive a cyan fluorescent protein. We demonstrate here that this is a useful tool for measuring the length of G2 phase without perturbing any aspect of cell cycle progression.


**Abstract** Patients with multiple myeloma (MM) who relapse after autologous transplantation have limited therapeutic options. We conducted a prospective, multicenter, phase IIa study to investigate the safety and efficacy of i.v. busulfan (Bu) in combination with bortezomib as a conditioning regimen for a second autotransplantation. Because a safe Bu exposure was unknown in patients receiving this combination, Bu was initially targeted to a total area under the concentration-time curve (AUC) of 20,000 μM × minute. As no concentration-limiting toxicity was observed in 6 patients, this Bu exposure was utilized in the following treatment cohort (n = 24). Individualized Bu dose, based on test dose 8 mg/kg pharmacokinetics (PK), was administered daily for 4 consecutive days starting 5 days before transplantation, followed by a single dose of bortezomib (1.3 mg/m2) 1 day before transplantation. The total mean dose of i.v. Bu (including the test dose and 4-day administration) was 14.2 mg/kg (standard deviation = 2.48; range, 8.7 to 19.2). Confirmatory PK demonstrated that only 2 of 30 patients who underwent transplantation were dose outside the Bu AUC target and dose adjustments were made for the last 2 doses of i.v. Bu. The median age was 59 years (range, 48 to 73). Median time from first to second transplantation was 28.0 months (range, 12 to 119). Of 26 evaluable patients, 10 patients attained a partial response (PR) or better at 3 months after
transplantation, with 2 patients attaining a complete response. At 6 months after transplantation, 5 of 12 evaluable patients had maintained or improved their disease status. Median progression-free survival was 197 days, whereas median overall survival was not reached during the study period. The most common grade 3 and 4 toxicities were febrile neutropenia (50.0%) and stomatitis (43.3%). One transplantation-related death was observed. A combination of dose-targeted i.v. Bu and bortezomib induced PR or better in one third of patients with MM who underwent a second autotransplantation, with acceptable toxicity.


**Disruption of heme-peptide cross-linking in mammalian peroxidases by hypochlorous acid.** J Inorg Biochem. 2014 Nov;140:245-54. Epub 2014 Jul 8. PMID: 25193127

Abstract  Myeloperoxidase (MPO), lactoperoxidase (LPO) and eosinophil peroxidase (EPO) play a central role in oxidative damage in inflammatory disorders by utilizing hydrogen peroxide and halides/pseudo halides to generate the corresponding hypohalous acid. The catalytic sites of these enzymes contain a covalently modified heme group, which is tethered to the polypeptide chain at two ester linkages via the methyl group (MPO, EPO and LPO) and one sulfonium bond via the vinyl group (MPO only). Covalent cross-linking of the catalytic site heme to the polypeptide chain is thought to play a protective role, since it renders the heme moiety less susceptible to the oxidants generated by these enzymes. Mass-spectrometric analysis revealed the following possible pathways by which hypochlorous acid (HOCl) disrupts the heme-protein cross-linking: (1) the methyl-ester bond is cleaved to form an alcohol; (2) the alcohol group undergoes an oxygen elimination reaction via the formation of an aldehyde intermediate or undergoes a demethylation reaction to lose the terminal CH2 group; and (3) the oxidative cleavage of the vinyl-sulfonium linkage. Once the heme moiety is released it undergoes cleavage at the carbon-methylene bridge either along the 5-β or a γ axis to form different pyrrole derivatives. These results indicate that covalent cross-linking is not enough to protect the enzymes from HOCl mediated heme destruction and free iron release. Thus, the interactions of mammalian peroxidases with HOCl modulates their activity and sets a stage for initiation of the Fenton reaction, further perpetuating oxidative damage at sites of inflammation.

Lonardo F, Guan H, Dzinic S, Sheng S.

**Maspin expression patterns differ in the invasive versus lepidic growth pattern of pulmonary adenocarcinoma.** Histopathology. 2014 Jul 5. PMID: 25040445

Abstract  AIMS: To test whether changes in the subcellular localization of maspin parallel morphological progression in pulmonary adenocarcinoma, we compared its expression between lepidic and invasive growth patterns.

METHODS: Applying immunohistochemistry, we compared maspin expression in lepidic and invasive growth patterns occurring in different tumors (series #1, n = 86) as well as within the same tumor and in the same section (series #2, n = 29).

RESULTS: In both series, the lepidic growth pattern (n = 45) was significantly associated with nuclear maspin, while the invasive (n = 70) with combined nuclear and cytoplasmic maspin (P < 0.05). In the second series, transition from a lepidic to an invasive pattern in the same tumor was associated predominantly with a shift respectively from a nuclear to a combined nuclear and cytoplasmic maspin (15/29) or preservation of nuclear expression (8/29). A shift from nuclear maspin to negative expression (3/29) or other patterns (3/29) were also observed.

CONCLUSIONS: Nuclear maspin is a typical but not exclusive feature of the lepidic growth pattern of pulmonary adenocarcinoma, whereas combined nuclear and cytoplasmic maspin characterizes invasion. These data show that changes of expression and subcellular localization of maspin may constitute an important biological end point of tumour progression and aid in the classification of lung adenocarcinoma.

...and a nice splattering of collaboration among Drs. Stemmer (TBM), Mattingly (MT), Bollig Fischer (MT) and Boerner (MT)

Madden JM, Mueller KL, Bollig-Fischer A, Stemmer P, Mattingly RR, Boerner JL


Abstract  Triple-negative breast cancer (TNBC) patients suffer from a highly malignant and aggressive disease. They have a high rate of relapse and often develop resistance to standard chemotherapy. Many TNBCs have elevated epidermal growth factor receptor (EGFR) but are resistant to EGFR inhibitors as monotherapy. In this study, we sought to find a combination therapy that could sensitize TNBC to EGFR inhibitors. Phospho-mass spectrometry was performed on the TNBC cell line, BT20, treated with 0.5 μM gefitinib. Immunoblotting measured protein levels and phosphorylation. Colony formation and growth assays analyzed the treatment on cell proliferation, while MTT assays determined the synergistic effect of inhibitor combination. A Dual-Luciferase reporter gene plasmid measured translation. All statistical analysis was done on CalcuSyn and GraphPad Prism using ANOVAs. Phospho-proteomics identified the mTOR pathway to be of interest in EGFR inhibitor resistance. In our studies, combining gefitinib and temsirolimus decreased cell growth and survival in a synergistic manner. Our data identified eIF4B, as a potentially key fragile point in EGFR and mTOR inhibitor synergy. Decreased eIF4B phosphorylation correlated with drops in growth, viability, clonogenic survival, and cap-dependent translation. Taken together, these data suggest EGFR and mTOR inhibitors abrogate growth, viability, and survival via disruption of eIF4B phosphorylation leading to decreased translation in TNBC cell lines. Further, including an mTOR inhibitor along with an EGFR inhibitor in TNBC with increased EGFR expression should be further explored. Additionally, translational regulation may play an important role in regulating EGFR and mTOR inhibitor synergy and warrant further investigation.


Abstract  Advanced technologies and biomaterials developed for tissue engineering and regenerative medicine present tractable biomimetic systems with potential applications for cancer research. Recently, the National Cancer Institute convened a Strategic Workshop to explore the use of tissue biomimicking for development of dynamic, physiologically relevant in vitro and ex vivo biomimetic systems to study cancer biology and drug efficacy. The workshop provided a forum to identify current progress, research gaps, and necessary steps to advance the field. Opportunities discussed included development of tumor biomimetic systems with an emphasis on reproducibility and validation of new biomimetic tumor models, as described in this report.

Espinoza E, Hassan A, Vaishampayan U, Shi D, Pontes JE, Weaver DW
PMID: 25177547

Abstract  Renal cell carcinoma (RCC) has a potential to metastasize to almost any site and this may occur many years following nephrectomy. We present six cases with uncommon sites of metastasis: four patients presented with distal pancreatic metastasis and two with duodenal/head of the pancreas metastasis. Time to metastatic disease varied from 1 to 19 years following renal surgery. For patients are alive and two succumbed to their disease. Long-term survival can be achieved with aggressive surgical excision of disease.

...from Drs. Wei and Cher with Dr. Littrup (MI)

Veenstra JJ, Gibson HM, Littrup PJ, Reyes JD, Cher ML, Takashima A, Wei WZ.
Cryotherapy with Concurrent CpG Oligonucleotide Treatment Controls Local Tumor Recurrence and Modulates HER2/neu Immunity. Cancer Res; 74(19); 1-12. PMID: 25092895

Abstract  Percutaneous cryoablation is a minimally invasive procedure for tumor destruction, which can potentially initiate or amplify antitumor immunity through the release of tumor-associated antigens. However, clinically efficacious immunity is lacking and regional recurrences are a limiting factor relative to surgical excision. To understand the mechanism of immune activation by cryoablation, comprehensive analyses of innate immunity and HER2/neu humoral and cellular immunity following cryoablation with or without peritumoral CpG injection were conducted using two HER2/neu+ tumor systems in wild-type (WT), neu-tolerant, and SCID mice. Cryoablation of neu+ TUBO tumor in BALB/c mice resulted in systemic immune priming, but not in neu-tolerant BALB NeuT mice. Cryoablation of human HER2+ D2F2/E2 tumor enabled the functionality of tumor-induced immunity, but secondary tumors were refractory to antitumor immunity if rechallenge occurred during the resolution phase of the cryoablated tumor. A step-wise increase in local recurrence was observed in WT, neu-tolerant, and SCID mice, indicating a role of adaptive immunity in controlling residual tumor foci. Importantly, local recurrences were eliminated or greatly reduced in WT, neu tolerant, and SCID mice when CpG was incorporated in the cryoablation regimen, showing significant local control by innate immunity. For long-term protection, however, adaptive immunity was required because most SCID mice eventually succumbed to local tumor recurrence even with combined cryoablation and CpG treatment. This improved understanding of the mechanisms by which cryoablation affects innate and adaptive immunity will help guide appropriate combination of therapeutic interventions to improve treatment outcomes.

...a remarkable finding using a component from Sucrets....a mainstay in family medicine cabinets for decades !! Dr. Xie extends his previous studies of dyclonine.

Ju D, Xie Y.

Abstract  The proteasome has become an important target for cancer therapy with the approval of bortezomib for the treatment of relapsed/refractory multiple myeloma (MM). However, numerous patients with MM do not respond to bortezomib and those responding initially often acquire resistance. Recent clinical studies have also demonstrated that bortezomib is also ineffective in treatment of other types of cancer. Therefore, it is imperative to develop novel approaches and agents for proteasome-targeting cancer therapy. In the present study, it was revealed that dyclonine, a major component of the cough droplets Sucrets, markedly enhances the cytotoxic effects of bortezomib and minimizes drug resistance in MM cells. It was demonstrated that a combination of bortezomib and dyclonine markedly induced apoptosis of MM cells. The present study suggests a novel therapeutic use of an over-the-counter medicine for the treatment of MM.

...and Dr. Hillman continues her collaborative work with Dr. Haacke (MI)


Abstract  PURPOSE/OBJECTIVE(S): Angiogenic blockade with irradiation may enhance the therapeutic ratio of radiation therapy (RT) through vascular normalization. We sought to determine the safety and toxicity profile of continuous daily-dosed sunitinib when combined with hypofractionated stereotactic RT (ISRT) for recurrent high-grade gliomas (rHGG). METHODS AND MATERIALS: Eligible patients had malignant high-grade glioma that recurred or progressed after primary surgery and RT. All patients received a minimum of a 10-day course of ISRT, had World Health Organization performance status of 0 to 1, and a life expectancy of >3 months. During ISRT, sunitinib was administered at 37.5 mg daily. The primary endpoint was acute toxicity, and response was assessed via serial magnetic resonance imaging. RESULTS: Eleven patients with rHGG were enrolled. The ISRT doses delivered ranged from 30 to 42 Gy in 2.5- to 3.75-Gy

Abstract Diabetes increases oxidative stress in the retina and decreases the levels of the intracellular antioxidant glutathione (GSH). The transcriptional factor Nrf2 regulates the expression of Gclc, the enzyme important in the biosynthesis of GSH, and in diabetes the binding of Nrf2 at the antioxidant response element region 4 (ARE4) is decreased. Our aim was to investigate the role of epigenetic modifications in the decreased Nrf2 binding at Gclc-ARE4 in the development of diabetic retinopathy and in the metabolic memory associated with its continued progression. The effect of hyperglycemia on H3K4 methylation in Nrf2 binding at Gclc-ARE4 was investigated by chromatin immunoprecipitation in the rat retina and was confirmed in retinal endothelial cells in which histone demethylase (LSD1) was manipulated. The role of histone methylation at Gclc-ARE4 in the metabolic memory was examined in rats maintained under poor control for 3 months followed by good control (GC) for 3 months. Although H3K4me2 at Gclc-ARE4 was increased in diabetes, H3K4me3 and H3K4me1 were decreased. LSD1 siRNA abrogated the glucose-induced decrease in H3K4me1 at Gclc-ARE4 and ameliorated decreases in Nrf2 binding at Gclc-ARE4 and Gclc transcripts. Reestablishment of GC failed to provide any benefits to histone methylation, and Nrf2 binding activity remained compromised. Thus, in diabetic retinopathy, histone methylation at Gclc-ARE4 plays an important role in regulating the Nrf2-Gclc-GSH cascade. Targeting histone methylation could help inhibit/slow down this binding disease.


Abstract The actin-bundling protein TRIOBP-4 and -5 promotes the motility of pancreatic cancer cells. TRIOBP isoforms 4 and 5 (TRIOBP-4/5) are an actin-bundling protein associated with hearing loss. Here, we showed that TRIOBP-4/5 was up-regulated in human pancreatic carcinoma cells. Knockdown of TRIOBP-4/5 led to a loss of filopodia and a decrease in cell motility. Confocal microscopy showed that re-expression of GFP-TRIOBP-4 or -5 restored the filopodial formation in TRIOBP-4/5-deficient PAN-C1 cells. Finally, TRIOBP-4/5 was shown to be overexpressed in human pancreatic cancer tissues. These results demonstrate a novel role of TRIOBP-4/5 that promotes the motility of pancreatic cancer cells via regulating actin cytoskeleton reorganization in the filopodia of the cells.


Abstract Ipilimumab augments antitumor activity of bispecific antibody-armed T cells. Ipilimumab is an agonistic monoclonal antibody against cytotoxic T-lymphocyte antigen-4 (CTLA-4) that enhances antitumor immunity by inhibiting immunosuppressive activity of regulatory T cells (Treg). In this study, we investigated whether inhibiting Treg activity with ipilimumab during ex vivo T cell expansion could augment anti-CD3-driven T cell proliferation and enhance bispecific antibody (BiAb)-redirected antitumor cytotoxicity of activated T cells (ATC). METHODS: PBMC from healthy individuals were stimulated with anti-CD3 monoclonal antibody with or without ipilimumab and expanded for 10-14 days. ATC were harvested and armed with anti-CD3 x anti-EGFR BiAb (EGFRBi) or anti-CD3 x anti-CD20 BiAb (CD20Bi) to test for redirected cytotoxicity against COLO356/FG pancreatic cancer cell line or Burkitt's lymphoma cell line (Daudi). RESULTS: In PBMC from healthy individuals, the addition of ipilimumab at the initiation of culture significantly enhanced T cell proliferation (p = 0.0029). ATC grown in the presence of ipilimumab showed significantly increased mean tumor-specific cytotoxicity at effector:target (E:T) ratio of 25:1 directed at COLO356/FG and Daudi by 37.71% (p < 0.0004) and 27.5% (p < 0.0004), respectively, and increased the secretion of chemokines (CCL2, CCL3, CCL4, CCL5, CXCL9, and granulocyte-macrophage colony stimulating factor (GM-CSF)) and cytokines (IFN-γ, IL-2R, IL-12, and IL-13), while reducing IL-10 secretion. CONCLUSIONS: Expansion of ATC in the presence of ipilimumab significantly improves not only the T cell proliferation but it also enhances cytokine secretion and the specific cytotoxicity of T cells armed with bispecific antibodies.


Abstract Omega-3 fatty acids have been reported to inhibit the growth of primary tumors, but their potential as a preventive agent for recurring cancers is unexplored. The primary objectives of this investigation are to examine whether eicosapentaenoic acid (EPA; one of the ω-3 PUFA) synergizes with FuOx (5-FU+Oxaliplatin), the backbone of colon cancer chemotherapy, and (b) whether EPA by itself or in combination with conventional chemotherapy prevents the recurrence of colon cancer via eliminating/suppressing CSCs/CSCCs. FuOx-resistant (chemo-resistant; CR) colon cancer cells, highly enriched in CSCs, were utilized for this study. While EPA alone was effective, combination of EPA and FuOx was more potent in (a) inhibiting cell growth,
colonosphere formation and sphere-forming frequency, (b) increasing sphere disintegration, (c) suppressing the growth of SCID mice xenografts of CR colon cancer cells, and (d) decreasing pro-inflammatory metabolites in mice. Additionally, EPA + FuOx caused a reduction in CSC/CSLC population. The growth reduction by this regimen is the result of increased apoptosis as evidenced by PARP cleavage. Furthermore, increased pPTEN, decreased pAkt, normalization of β-catenin expression, localization and transcriptional activity by EPA suggests a role for PTEN/Akt axis and Wnt signaling in regulating this process. Our data suggest that EPA by itself or in combination with FuOx could be an effective preventive strategy for recurring CRC.

... a timely publication from Dr. Neely using a zebrafish model and just following Dr. Parajuli's Program meeting talk on Sep 8 which described studies with zebrafish...

Rowe HM, Withey JH, Neely MN. 
Zebrafish as a model for zoonotic aquatic pathogens. 
PMID: 24607289

Abstract 
Aquatic habitats harbor a multitude of bacterial species. Many of these bacteria can act as pathogens to aquatic species and/or non-aquatic organisms, including humans, that come into contact with contaminated water sources or colonized aquatic organisms. In many instances, the bacteria are not pathogenic to the aquatic species they colonize and are only considered pathogens when they come into contact with humans. There is a general lack of knowledge about how the environmental lifestyle of these pathogens allows them to persist, replicate and produce the necessary pathogenic mechanisms to successfully transmit to the human host and cause disease. Recently, the zebrafish infectious disease model has emerged as an ideal system for examining aquatic pathogens, both in the aquatic environment and during infection of the human host. This review will focus on how the zebrafish has been used successfully to analyze the pathogenesis of aquatic bacterial pathogens.

Wang NY, Patras KA, Seo HS, Cavaco CK, Rösler B, Neely MN, Sullam PM, Doran KS. 
Group B streptococcal serine-rich repeat proteins promote interaction with fibrinogen and vaginal colonization. 
PMID: 24620021

Abstract 
Group B streptococcus (GBS) can cause severe disease in susceptible hosts, including newborns, pregnant women, and the elderly. GBS serine-rich-repeat (Srr) surface glycoproteins are important adhesins/invasins in multiple host tissues, including the vagina. However, exact molecular mechanisms contributing to their importance in colonization are unknown. We have recently determined that Srr proteins contain a fibrinogen-binding region (BR) and hypothesize that Srr-mediated fibrinogen binding may contribute to GBS cervicovaginal colonization. In this study, we observed that fibrinogen enhanced wild-type GBS attachment to cervical and vaginal epithelium, and that this was dependent on Srr1. Moreover, purified Srr1-BR peptide bound directly to host cells, and peptide administration in vivo reduced GBS recovery from the vaginal tract. Furthermore, a GBS mutant strain lacking only the Srr1 "latching" domain exhibited decreased adhesion in vitro and decreased persistence in a mouse model of GBS vaginal colonization, suggesting the importance of Srr-fibrinogen interactions in the female reproductive tract.

Safety and Efficacy of Targeted-Dose Busulfan and Bortezomib as a Conditioning Regimen for Patients with Relapsed Multiple Myeloma Undergoing a Second Autologous Blood Progenitor Cell Transplantation. 
PMID: 25139216

Abstract 
Patients with multiple myeloma (MM) who relapse after autologous transplantation have limited therapeutic options. We conducted a prospective, multicenter, phase IIa study to investigate the safety and efficacy of i.v. busulfan (Bu) in combination with bortezomib as a conditioning regimen for a second autotransplantation. Because a safe Bu exposure was unknown in patients receiving this combination, Bu was initially targeted to a total area under the concentration-time curve (AUC) of 20,000 µM × minute. As no concentration-limiting toxicity was observed in 6 patients, this Bu exposure was utilized in the following treatment cohort (n = 24). Individualized Bu dose, based on a test dose 8 mg/kg pharmacokinetics (PK), was administered daily for 4 consecutive days starting 5 days before transplantation, followed by a single dose of bortezomib (1.3 mg/m²) 1 day before transplantation. The total mean dose of i.v. Bu (including the test dose and 4-day administration) was 14.2 mg/kg (standard deviation = 2.48; range, 8.7 to 19.2). Confirmatory PK demonstrated that only 2 of 30 patients who underwent transplantation were dose outside the Bu AUC target and dose adjustments were made for the last 2 doses of i.v. Bu. The median age was 59 years (range, 48 to 73). Median time from first to second transplantation was 28.0 months (range, 12 to 119). Of 26 evaluable patients, 10 patients attained a partial response (PR) or better at 3 months after transplantation, with 2 patients attaining a complete response. At 6 months after transplantation, 5 of 12 evaluable patients had maintained or improved their disease status. Median progression-free survival was 191 days, whereas median overall survival was not reached during the study period. The most common grade 3 and 4 toxicities were febrile neutropenia (50.0%) and stomatitis (43.3%). One transplantation-related death was observed. A combination of dose-targeted i.v. Bu and bortezomib induced PR or better in one third of patients with MM who underwent a second autotransplantation, with acceptable toxicity.

Abstract Myeloperoxidase (MPO), lactoperoxidase (LPO) and eosinophil peroxidase (EPO) play a central role in oxidative damage in inflammatory disorders by utilizing hydrogen peroxide and halides/pseudo halides to generate the corresponding hypohalous acid. The catalytic sites of these enzymes contain a covalently modified heme group, which is tethered to the polypeptide chain at two ester linkages via the methyl group (MPO, EPO and LPO) and one sulfonium bond via the vinyl group (MPO only). Covalent cross-linking of the catalytic site heme to the polypeptide chain in peroxidases is thought to play a protective role, since it renders the heme moiety less susceptible to the oxidants generated by these enzymes. Mass-spectrometric analysis revealed the following possible pathways by which hypochlorous acid (HOCl) disrupts the heme-protein cross-linking: (1) the methyl-ester bond is cleaved to form an alcohol; (2) the alcohol group undergoes an oxygen elimination reaction via the formation of an aldehyde intermediate or undergoes a demethylation reaction to lose the terminal CH2 group; and (3) the oxidative cleavage of the vinyl-sulfonium linkage. Once the heme moiety is released it undergoes cleavage at the carbon-methylene bridge either along the ß- or a ß axis to form different pyrrole derivatives. These results indicate that covalent cross-linking is not enough to protect the enzymes from HOCl mediated heme destruction and free iron release. Thus, the interactions of mammalian peroxidases with HOCl modulates their activity and sets a stage for initiation of the Fenton reaction, further perpetuating oxidative damage at sites of inflammation.


Abstract Advanced technologies and biomaterials developed for tissue engineering and regenerative medicine present tractable biomimetic systems with potential applications for cancer research. Recently, the National Cancer Institute convened a Strategic Workshop to explore the use of tissue biomanufacturing for development of dynamic, physiologically relevant in vitro and ex vivo biomimetic systems to study cancer biology and drug efficacy. The workshop provided a forum to identify current progress, research gaps, and necessary steps to advance the field. Opportunities discussed included development of tumor biomimetic systems with an emphasis on reproducibility and validation of new biomimetic tumor models, as described in this report.

...two new manuscripts from Dr. Todi

Abstract Polyglutamine repeat expansion in ataxin-3 causes neurodegeneration in the most common dominant ataxia, spinocerebellar ataxia type 3 (SCA3). Since reducing levels of disease proteins improves pathology in animals, we investigated how ataxin-3 is degraded. Here we show that, unlike most proteins, ataxin-3 turnover does not require its ubiquitination, but is regulated by ubiquitin-binding site 2 (UbS2) on its N terminus. Mutating UbS2 decreases ataxin-3 protein levels in cultured mammalian cells and in Drosophila melanogaster by increasing its proteasomal turnover. Ataxin-3 interacts with the proteasome-associated proteins Rad23A/B through UbS2. Knockdown of Rad23 in cultured cells and in Drosophila results in lower levels of ataxin-3 protein. Importantly, reducing Rad23 suppresses ataxin-3-dependent degeneration in flies. We present a mechanism for ubiquitination-independent degradation that is impeded by protein interactions with proteasome-associated factors. We conclude that UbS2 is a potential target through which to enhance ataxin-3 degradation for SCA3 therapy.

Ristic G, Tsou WL, Todi SV.

Abstract The Ubiquitin-Proteasome Pathway (UPP), which is critical for normal function in the nervous system and is implicated in various neurological diseases, requires the small modifier protein ubiquitin to accomplish its duty of selectively degrading short-lived, abnormal or misfolded proteins. Over the past decade, a large class of proteases collectively known as deubiquitinating enzymes (DUBs) has increasingly gained attention in all manners related to ubiquitin. By cleaving ubiquitin from another protein, DUBs ensure that the UPP functions properly. DUBs accomplish this task by processing newly translated ubiquitin so that it can be used for conjugation to substrate proteins, by regulating the "where, when, and why" of UPP substrate ubiquitination and subsequent degradation, and by recycling ubiquitin for re-use by the UPP. Because of the reliance of the UPP on ubiquitin, it is not surprising that these proteases play important roles in the normal activities of the nervous system and in neurodegenerative diseases. In this review, we summarize recent advances in understanding the functions of DUBs in the nervous system. We focus on their role in the UPP, and make the argument that understanding the UPP from the perspective of DUBs can yield new insight into diseases that result from anomalous intra-cellular processes or inter-cellular networks. Lastly, we discuss the relevance of DUBs as therapeutic options for disorders of the nervous system.

Espinoza E, Hassan A, Vaishampayan U, Shi D, Pontes JE, Weaver DW

Abstract Renal cell carcinoma (RCC) has a potential to metastasize to almost any site and this may occur many years following nephrectomy. We present six cases with uncommon sites of metastasis: four patients presented with distal pancreatic metastasis and two with duodenal/head of the pancreas metastasis. Time to metastatic disease varied from 1 to 19 years following renal surgery. For patients are alive and two succumbed to their disease. Long-term survival can be achieved with aggressive surgical excision of disease.

Approximately June 25 2014 – September 24, 2014