New Funding $$

1. **Dr. Lawrence G. Lum** received funding support from the Solving Kids’ Cancer Therapeutic Development Initiative for his proposal “Activated T Cells Armed With GD2 Bispecific Antibody”.

2. **Lauren Tanabe** received a two year postdoctoral fellowship to study the role of matriptase in inflammatory breast cancer from the American Cancer Society. Lauren is working and studying in Dr. Karin List’s laboratory.

3. **Dr. Rafi Fridman** received a new R21 from the National Cancer Institute for his work with focuses on the roles of discoidin domain receptors in pancreatic pre-neoplasia.

Accolades

Poster Winners – Tumor Biology and Microenvironment Program Retreat December 12, 2014

1. **Carlos Redondo Murcia** won first place for his poster titled “Towards a stem cell compliant far-red trackable Tet-On technology at our Tumor Biology and Microenvironment Program Retreat. Carlos is working and studying in Dr. Rodrigo Fernandez-Valdivia’s laboratory.

2. **Roselyne Labbe’** won second place for her poster titled “Dexamethasone variably protects triple negative breast cancer cells against paclitaxel depending on relative cellular levels of glucocorticoid receptor alpha. Roselyne is working and studying in Dr. Cecilia Speyer’s laboratory.

Hot off the Press –

**SELECTION OF PUBLICATIONS**

Koh M, Woo Y, Valiathan RR, Jung HY, Park SY, Kim YN, **Kim HR, Fridman R**, Moon A.  
**Discoidin domain receptor 1 is a novel transcriptional target of ZEB1 in breast epithelial cells undergoing H-Ras-induced epithelial to mesenchymal transition.** Int J Cancer. 2014 Aug 23. **PMID: 25155634**

Abstract  The epithelial-to-mesenchymal transition (EMT) process allows carcinoma cells to dissociate from the primary tumor thereby facilitating tumor cell invasion and metastasis. Ras-dependent hyperactive signaling is commonly associated with tumorigenesis, invasion, EMT, and metastasis. However, the downstream effectors by which Ras regulates EMT remain ill defined. In this study, we show that the H-Ras pathway leads to mesenchymal-like phenotypic changes in human breast epithelial cells by controlling the ZEB1/microRNA-200c axis. Moreover, H-Ras suppresses the expression of the discoidin domain receptor 1 (DDR1), a collagen receptor tyrosine kinase, via ZEB1, thus identifying ZEB1 as a novel transcriptional repressor of DDR1. Mutation studies on the putative promoter of the DDR1 gene revealed that bipartite Z- and E-box elements play a key role in transcriptional repression of DDR1 in Hs578T and MDA-MB-231 breast carcinoma cell lines by ZEB1. Furthermore, we found an inverse correlation between ZEB1 and DDR1 expression in various cancer cell lines and in human breast carcinoma tissues. Consistently, overexpression of DDR1 reduced the invasive phenotype of mesenchymal-like triple-negative breast cancer cells in 3D cultures and in vivo. Thus, ZEB1’s role in maintenance of EMT in breast carcinoma cells is mediated in part by its ability to suppress DDR1 expression and consequently contribute to the activation of the invasive phenotype. Taken together, our results unveil a novel H-Ras/ZEB1/DDR1 network that contributes to breast cancer progression in triple-negative breast cancers.


Abstract  Adipocyte lipolysis can increase the production of inflammatory cytokines such as interleukin-6 (IL-6) that promote insulin resistance. However, the mechanisms that link lipolysis with inflammation remain elusive. Acute activation of β3-adrenergic receptors (ADR3) triggers lipolysis and up-regulates production of IL-6 in adipocytes, and both of these effects are blocked by pharmacological inhibition of hormone-sensitive lipase. We report that stimulation of ADR3 induces expression of sphingosine kinase 1 (SphK1) and increases sphingosine 1-phosphate production in adipocytes in a manner that also depends on hormone-sensitive lipase activity. Mechanistically, we found that adipose lipolysis-induced SphK1 up-regulation is mediated by the c-Jun N-terminal kinase (JNK)/activating protein-1 signaling pathway. Inhibition of SphK1 by sphingosine kinase inhibitor 2 diminished the ADR3-induced IL-6 production both in vitro and in vivo. Induction of IL-6 by ADR3 activation was suppressed by siRNA knockdown of Sphk1 in cultured adipocytes and was
severely attenuated in Sphk1 null mice. Conversely, ectopic expression of SphK1 increased IL-6 expression in adipocytes. Collectively, these data demonstrate that SphK1 is a critical mediator in lipolysis-triggered inflammation in adipocytes.

…Four collaborative papers from Dr. Ali-Fehmi…two with our newer member Dr. Bandyopadhyay


Abstract The incidence of endometrial cancers diagnosed on biopsy that have no residual cancer identified at hysterectomy is not well studied. The aim of our study was to determine the incidence and long-term follow-up of this “vanishing cancer” phenomenon. All slides from the initial biopsy/curettage and hysterectomy specimens were reviewed and the diagnosis confirmed by a gynecologic pathologist. The entire endometrium was serially sectioned and submitted for histologic examination. Clinical and pathologic variables were analyzed, including patient demographics, tumor histologic type and grade, stage, biopsy method, adjuvant therapy, surgical procedure, recurrence, and disease-specific survival. We identified 23 biopsy-proven cases of endometrial cancer with no residual disease on hysterectomy specimen. Of the 23 patients, 15 (65.2%) were diagnosed as endometriod, 6 (26%) serous, 1 clear cell (4.3%), and 1 (4.3%) serous intraepithelial carcinoma. Seventeen underwent dilatation and curettage, and 6 had endometrial biopsy as the primary procedure. The median follow-up was 8.8 years (range, 1.2 to 17 y). Only 2 cases with serous carcinoma underwent adjuvant chemotherapy, and none received radiation therapy. Only 1 patient died of disease after 27 months and was diagnosed as FIGO grade II endometrioid carcinoma on dilatation and curettage. The inability to identify cancer in a hysterectomy specimen for biopsy-confirmed carcinoma does not indicate technical failure. Although there is no specific standard treatment for patients with “vanishing endometrial cancer,” the prognosis is excellent; however, close follow-up is suggested.


Abstract PURPOSE: Objective(s): There is paucity of data in regards to prognostic factors and outcome of women with 2009 FIGO stage II disease. The objective of this study was to investigate prognostic factors, recurrence patterns and survival endpoints in this group of patients. MATERIALS: Methods: Data from four academic institutions were analyzed. 130 women were identified with 2009 FIGO stage II. All patients underwent hysterectomy, oophorectomy and lymph node evaluation with or without pelvic and paraaortic lymph node dissection and peritoneal cytology. The Kaplan-Meier approach and Cox regression analysis were used to estimate recurrence-free (RFS), disease-specific (DSS) and overall survival (OS). RESULTS: Median follow-up was 44months. 120 patients (92%) underwent simple hysterectomy, 78% had lymph node dissection and 95% had peritoneal cytology examination. 99 patients (76%) received adjuvant radiation treatment (RT). 5-year RFS, DSS and OS was 77%, 90%, and 72%, respectively. On multivariate analysis of RFS, adjuvant RT, the presence of lymphovascular space invasion (LVI) and high tumor grades were significant predictors. For DSS, LVI and high tumor grades were significant predictors while older age and high tumor grade were the only predictors of OS. CONCLUSIONS: In this multi-institutional study, disease-specific survival for women with FIGO stage II uterine endometrioid carcinoma is excellent. High tumor grade, lymphovascular space involvement, adjuvant radiation treatment and old age are important prognostic factors. There was no significant difference in outcome between patients who received vaginal cuff brachytherapy compared to those received pelvic external beam radiation treatment.


Abstract To analyze the clinical significance of the extent of lymphovascular space invasion (LVI) in patients with uterine serous carcinoma. After IRB approval, 232 patients with uterine serous carcinoma from the pathology databases of 4 large academic institutions were included. Patients were divided into 3 groups based on extent of LVI. Extensive LVI (E-LVI) was defined as ≥3 vessel involvement; low LVI (L-LVI) was defined <3 vessel involvement; and the third group consisted of tumors with no LVI (A-LVI). The association between LVI and myometrial invasion, cervical involvement, lower uterine segment involvement, positive peritoneal washings, lymph node involvement, stage, and survival were analyzed. Of 232 patients, 47 had E-LVI (20.3%), 83 had L-LVI (35.8%), and 102 had A-LVI (44%). A total of 9.8% of the patients with A-LVI had lymph node involvement as compared with 18.1% in the L-LVI group and 55.4% in the E-LVI group (P<0.0001). Fifty-nine percent of the patients in A-LVI, 85% in L-LVI, and 100% in the E-LVI group demonstrated myometrial invasion (P=0.0001). Cervical involvement was noted in 23%, 43%, 66% (P<0.0001) and lower uterine segment involvement in 31%, 43%, and 42% of A-LVI, L-LVI, and E-LVI (P<0.0001), respectively. Stage III and IV disease were seen in 29%, 38%, and 79% of the patients with A-LVI, L-LVI, and E-LVI, respectively (P<0.0001). The median overall survival was 172, 95, and 39 mo for the A-LVI, L-LVI, and E-LVI groups, respectively (P=0.0001). The racial distribution was significant with African American patients demonstrating significantly more L-LVI (27.8%) and E-LVI (40.4%) when compared with A-LVI (19.6%) (P=0.040). In a subgroup analysis including patients with Stage I and II (n=123) revealed median survivals of 172, 169, and 38 mo in the A-LVI, L-LVI, and E-LVI groups, respectively (P=0.0001). Fifty percent of these patients with E-LVI, 20% in L-LVI group, and 15% in A-LVI group had disease recurrence (P=0.004). The extent of LVI was associated with multiple pathologic factors and was found to be a negative prognostic factor for overall survival and disease recurrence.


Abstract  Benign breast disease (BBD) is a very common condition, diagnosed in approximately half of all American women throughout their lifespan. White 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.


Atypical cell cycle control over neural cell fate. NO ABSTRACT AVAILABLE


Abstract  Human first-trimester trophoblast cells proliferate at low O2, but survival is compromised by oxidative stress, leading to uteroplacental insufficiency. The vasoactive drug, sildenafil citrate (Viagra, Sigma, St Louis, Missouri), has proven useful in reducing adverse pregnancy outcomes. An important biological function of this pharmaceutical is its action as an inhibitor of cyclic guanosine monophosphate (cGMP) phosphodiesterase type 5 activity, which suggests that it could have beneficial effects on trophoblast survival. To investigate whether sildenafil can prevent trophoblast cell death, human first-trimester villous explants and the HTR-8/SVneo cytotrophoblast cell line were exposed to hypoxia and reoxygenation (H/R) to generate oxidative stress, which induces apoptosis. Apoptosis was optimally inhibited during H/R by 350 ng/mL sildenafil. Sildenafil-mediated survival was reversed by l-NG-nitro-l-arginine methyl ester hydrochloride or cGMP antagonist, indicating a dependence on both nitric oxide (NO) and cGMP. Indeed, either a cGMP agonist or an NO generator was cytotoxic independent of sildenafil. These findings suggest a novel intervention route for patients with recurrent pregnancy loss or obstetrical placental disorders.


Abstract  The goal of the current study is to examine the biological effects of epithelial-specific tumor suppressor maspin on tumor host immune response. Accumulated evidence demonstrates an anti-tumor effect of maspin on tumor growth, invasion and metastasis. The molecular mechanism underlying these biological functions of maspin is thought to be through histone deacetylase inhibition, key to the maintenance of differentiated epithelial phenotype. Since tumor-driven stromal reactivities co-evolve in tumor progression and metastasis, it is not surprising that maspin expression in tumor cells inhibits extracellular matrix degradation, increases fibrosis and blocks hypoxia-induced angiogenesis. Using the athymic nude mouse model capable of supporting the growth and progression of xenogeneic human prostate cancer cells, we further demonstrate that maspin expression in tumor cells elicits neutrophil- and B cells-dependent host tumor immunogenicity. Specifically, mice bearing maspin-expressing tumors exhibited increased systemic and intratumoral neutrophil maturation, activation and antibody-dependent cytotoxicity, and decreased peritumoral lymphangiogenesis. These results reveal a novel biological function of maspin in directing host immunity towards tumor elimination that helps explain the significant reduction of xenograft tumor incidence in vivo and the clinical correlation of maspin with better prognosis of several types of cancer. Taken together, our data raised the possibility for novel maspin-based cancer immunotherapies.


Approximately September 24, 2014 – December 22, 2014
incidence of CMV viremia with relation to donor (D) and recipient (R) CMV serostatus were related donor transplantations, and 65% were unrelated donor transplantations. A total of 114 (45%) patients developed CMV viremia at a median of 34 days (range, 14 to 236 days) after transplantation. Only recipient CMV IgG serostatus was significantly associated with development of CMV viremia (P < .001). The incidence of CMV viremia with relation to donor (D) and recipient (R) CMV serostatus subgroups was as follows: D+/R+, 73%; D-/R+, 67%; D+/R-, 19%; and D-/R-. 0. A total of 31 patients were diagnosed with a biopsy-proven CMV gastroenteritis; 2 patients had evidence of CMV gastroenteritis and GVHD on the first biopsy and 29 on the second biopsy. Median time to development of CMV gastroenteritis was 52 days (range, 19 to 236 days) after transplantation. Using death as a competing risk, the cumulative incidence of CMV gastroenteritis at 1 year was 16.4%. The incidence of CMV gastroenteritis in relation to the donor/recipient serostatus was as follows: D+/R+, 22%; D-/R+, 31%; D+/R-, 12%; and D-/R-. 0. Median follow-up time for the 252 patients was 35.4 (95% CI 23.8 to 44.8) months. The estimated overall survival rate at 1 and 2 years was .45 (95% confidence interval [CI], .39 to .52) and .39 (95% CI .33 to .46), respectively. Of the examined variables, those related to the overall survival were maximal clinical GVHD grade (P < .001) and development of CMV gastroenteritis (P = .008). Development of CMV viremia was not associated with increased mortality. In conclusion, CMV gastroenteritis is common complication in patients with GI GVHD and can adversely affect the prognosis.


Abstract Gastrointestinal (GI) graft-versus-host disease (GVHD) is one of the most common causes of morbidity and mortality after allogeneic stem cell transplantation. In addition, cytomegalovirus (CMV) infection of the gastrointestinal tract can complicate the post-transplantation course of these patients and it can be difficult to differentiate the 2 diagnoses given that they can present with similar symptoms. We retrospectively analyzed 252 patients who were diagnosed with GI GVHD to evaluate the incidence, risk factors, and outcomes of CMV viremia and CMV gastroenteritis in these patients. The median age at the time of transplantation was 51 years, 35% were related donor transplantations, and 65% were unrelated donor transplantations. A total of 114 (45%) patients developed CMV viremia at a median of 34 days (range, 14 to 236 days) after transplantation. Only recipient CMV IgG serostatus was significantly associated with development of CMV viremia (P < .001). The incidence of CMV viremia with relation to donor (D) and recipient (R) CMV serostatus subgroups was as follows: D+/R+, 73%; D-/R+, 67%; D+/R-, 19%; and D-/R-. 0. A total of 31 patients were diagnosed with a biopsy-proven CMV gastroenteritis; 2 patients had evidence of CMV gastroenteritis and GVHD on the first biopsy and 29 on the second biopsy. Median time to development of CMV gastroenteritis was 52 days (range, 19 to 236 days) after transplantation. Using death as a competing risk, the cumulative incidence of CMV gastroenteritis at 1 year was 16.4%. The incidence of CMV gastroenteritis in relation to the donor/recipient serostatus was as follows: D+/R+, 22%; D-/R+, 31%; D+/R-, 12%; and D-/R-. 0. Median follow-up time for the 252 patients was 35.4 (95% CI 23.8 to 44.8) months. The estimated overall survival rate at 1 and 2 years was .45 (95% confidence interval [CI], .39 to .52) and .39 (95% CI .33 to .46), respectively. Of the examined variables, those related to the overall survival were maximal clinical GVHD grade (P < .001) and development of CMV gastroenteritis (P = .008). Development of CMV viremia was not associated with increased mortality. In conclusion, CMV gastroenteritis is common complication in patients with GI GVHD and can adversely affect the prognosis.


Abstract Hematopoietic cell transplantation (HCT) represents the most common and effective form of immunotherapy for childhood malignancies. The role of the graft-versus-leukemia effect in allogeneic HCT has been well established in childhood malignancies, but is also associated with short-term and long-term morbidity. HCT may be ineffective in some settings at obtaining control of the malignancy, and as such, cannot be used as a universal cancer immunotherapy. Novel therapies using dendritic cell vaccinations, tumor-infiltrating lymphocytes, and chimeric antigen receptor T cells are being evaluated as potential adjuvants to HCT.


Abstract BackgroundRedirection of T lymphocytes against tumor antigens can induce dramatic regression of advanced stage malignancy. The use of bispecific antibodies (BsAbs) that bind both the T-cell receptor (TCR) and a target antigen is one promising approach to T-cell redirection. However, BsAbs indiscriminately bind all CD3+ T-cells and trigger TCR activation in the absence of parallel costimulatory signals required to overcome T-cell unresponsiveness or anergy. MethodsTo address these limitations, a combination platform was designed wherein a unique BsAbs referred to as frBsAbs exclusively engages T-cells engineered to express a novel chimeric receptor comprised of extracellular folate receptor fused to intracellular TCR and CD28 costimulatory signaling domains in tandem; a BsAbs-binding primary human T-cells expressing the BsAbs-IR was specifically redirected against CD20+ leukemic cells or HER2+ epithelial cancer cells, respectively, while non-engineered T-cells were not activated. Notably, elimination of the CD28 costimulatory domain from the BsAbs-IR led to reduced TCR activation and costimulatory signals to BsAbs-IR T-cells. ConclusionsIn summary, our results establish the proof of concept that the chimeric receptor T cells are being evaluated as potential adjuvants to HCT.


Abstract Posaconazole tablets, a new oral formulation of posaconazole, can be effective when given as antifungal prophylaxis to neutropenic patients at high risk for invasive fungal infection (e.g., those with acute myelogenous leukemia or myelodysplastic syndrome). Such effectiveness might be specifically important to patients with poor oral intake because of nausea, vomiting, or chemotherapy-associated mucositis. This was a prospective, global study in high-risk patients to characterize the pharmacokinetics and safety profile of posaconazole tablets and to identify the dose of posaconazole tablets that would provide exposure within a predefined range of exposures (steady-state average concentration [area under the concentration-time curve/24 h] of ≥500 ng/ml and ≤2,500 ng/ml in >90% of patients). The study evaluated two sequential dosing cohorts: 200 mg posaconazole once daily (n = 20) and 300 mg posaconazole once daily (n = 34) (both cohorts had a twice-daily loading dose on day 1) taken without regard to food intake during the neutropenic period for ≤28 days. The exposure target was reached (day 8) in 15 of 19 (79%) pharmacokinetic-evaluable patients taking 200 mg posaconazole once daily and in 31 of 32 (97%) patients taking 300 mg posaconazole once daily, 300 mg posaconazole once daily achieved the desired exposure target. Posaconazole tablets were generally well tolerated in high-risk neutropenic patients. (This study has been registered at ClinicalTrials.gov under registration no. NCT01777763.).

Chandrasekar P, Havlichek D, Johnson LB.


Abstract Recent match results from the National Resident Matching Program for the subspecialty of infectious diseases show an ongoing decline in the number of fellowship positions filled, and, more important, in the number of applicants, particularly from the pool of international medical graduates. The main reasons for this declining application rate are unclear; in the absence of hard data, we present our viewpoint on this issue. Difficulties in securing visas for permanent residency in the United States, perception of a limited job market, and the explosive growth in the number of hospitalist positions may be important contributing factors. Infectious Diseases Society of America members need to focus on medical students and medical residents in their formative years. We present potential solutions to this problem of declining interest in the field of infectious diseases.

Salimnia H, Fairfax MR, Chandrasekar PH


Abstract BACKGROUND: Cytomegalovirus (CMV) causes significant morbidity and mortality in solid organ and bone marrow transplant recipients. DNA vaccines can provide both humoral and cellular immunity without exposing immune-compromised persons to replication-competent CMV. METHODS: We studied the kinetics of CMV vaccine DNA in plasma. The samples were obtained from vaccine recipients who were enrolled in a double-blinded, placebo-controlled clinical trial of an intramuscular, plasmid-based, bivalent DNA vaccine for CMV in stem cell transplant recipients. Residual specimens on patients enrolled in the vaccine trial were saved until the trial was unblinded and published. Quantitative real-time polymerase chain reaction (PCR) was used to detect and quantify CMV glycoprotein B (gB) DNA in plasma from 4 recipients of the vaccine. The melting temperature of the vaccine gB amplicon was 62.4°C, compared to 68.8°C, which is seen with the wild-type virus. RESULTS: Sequence analysis revealed that there were 3 mismatches between the fluorescent resonance energy transfer probe and the vaccine DNA sequence. CONCLUSION: Because preemptive treatment of CMV disease in stem cell transplant patients is based on quantitative PCR analysis of viral sequences in plasma, it is important that vaccine sequences not be confused with those in wild-type virus. Confusion could lead to treatment with toxic medications, potentially compromising the transplant. Effects of PCR target choice and amplicon detection techniques on patient management and vaccine trials are discussed.

Ross I, Womble P, Ye J, Linsell S, Montie JE, Miller DC, Cher ML.


Abstract The Michigan Urological Surgery Improvement Collaborative (MUSIC) is a statewide consortium of 44 urology practices that aims to improve the quality of prostate cancer care in Michigan. As an initial priority, we examined patterns of care in the radiographic staging of men with newly diagnosed prostate cancer. We sought to determine whether collaborative-wide data review and performance feedback would reduce the rate of imaging among men with low risk prostate cancer. MATERIALS AND METHODS: Practices submitted standardized data, including use and results of staging computed tomography (CT) and bone scans, to a web-based clinical registry for all men with newly-diagnosed prostate cancer. We identified all low risk prostate cancer patients and compared patterns of imaging utilization before and after practice-level performance feedback and guideline review provided at collaborative-wide meetings. RESULTS: Within MUSIC, 813 patients were newly diagnosed with low risk prostate cancer during the 19 month study period. Of the 410 patients diagnosed in the pre-feedback period (Phase I), 15 (3.7%) and 21 (5.2%) patients had a bone and CT scan respectively. In 403 patients diagnosed after feedback (Phase II), radiographic staging was obtained in 5 (1.3%) (p=0.03) and 13 (3.2%) (p=0.17) patients for bone scan and CT scan, respectively. CONCLUSION: The overall rate of radiographic staging for newly diagnosed low risk prostate cancer was appropriately low. The rate of imaging decreased even further following collaborative education and performance feedback. MUSIC appears to be a successful tool for quality improvement, affecting practice patterns and increasing efficiency of care.

Wakeling EN, Nahhas FA, Feldman GL.

Approximately September 24, 2014 – December 22, 2014
**Extra alleles in FMR1 triple-primed PCR: artifact, aneuploidy, or somatic mosaicism?**  
**J Mol Diagn. 2014 Nov;16(6):689-96.**  
**PMID:** 25307758

**Abstract**  
Triple-primed PCR assays have become the preferred fragile X syndrome testing method. Using a commercially available assay, we detected a reproducible extra peak(s) in 0.5% of 13,161 clinical samples. The objectives of this study were to determine the cause of these extra peaks; to identify whether these peaks represent an assay specific artifact, an underlying chromosome aneuploidy, or somatic mosaicism; and to ascertain their clinical relevance. The presence of an extra allele(s) was confirmed by a laboratory-developed PCR, with sequencing of the FMR1 5’ UTR or Southern blot for some samples. The laboratory-developed procedure detected the extra allele(s) in 57 of 64 samples. Thus, we confirmed an extra peak, typically of lower abundance, in approximately 0.4% of all samples. Of these samples, 5 were from males and 52 were from heterozygous or homozygous females. Six patients likely had X chromosome aneuploidies. In 82.3% of samples, the extra allele had fewer repeats than the predominant allele(s). Additional alleles detected by FMR1 triple-primed PCR are not an assay-specific artifact and are likely due to X chromosome aneuploidies or somatic repeat instability. Additional normal alleles likely have no clinical significance for fragile X syndrome carrier or affected status. Extra alleles in individuals with normal karyotypes probably represent FMR1 somatic variation.

**Reporting genomic secondary findings: ACMG members weigh in.**  
**Genet Med. 2014 Nov 13.**  
**PMID:** 25394173

**Abstract**  
Purpose: The aim of this study was to survey American College of Medical Genetics and Genomics members about secondary findings from clinical genome-scale sequencing. Methods: A Web-based survey was mailed to 1,687 members of the American College of Medical Genetics and Genomics. Exploratory factor analysis identified underlying factors assessed by survey items. Linear regression assessed associations between factor scores and respondent characteristics. Results: The response rate was 29%. Four factors explained 51% of the survey variance: best practices, patient preferences, guidance, and informed consent. Most agreed with “best practice” items describing seeking and reporting of secondary findings as consistent with medical standards, having sufficient evidence, and, for adults, the benefits generally outweighing potential harms. There was lack of agreement regarding benefits versus harms for children and impact on health-care resources. The majority agreed that patient preferences should be considered, including ability to opt out, and that informed consent was feasible and critical. Characteristics significantly associated with factor scores included country of residence, sequencing experience, and years in practice. Conclusion: The American College of Medical Genetics and Genomics should update a list of genes to be assessed when clinical genome-scale sequencing is performed. Informed consent is necessary, and reporting of secondary findings should be optional. Research on implementation of secondary findings reporting is needed.

**Lee YH, Pelkova AP, Konkar AA, Granneman JG.**  
**Cellular origins of cold-induced brown adipocytes in adult mice.**  
**FASEB J. 2014 Nov 12.**  
**PMID:** 25392270

**Abstract**  
This work investigated how cold stress induces the appearance of brown adipocytes (BAs) in brown and white adipose tissues (WATs) of adult mice. In interscapular brown adipose tissue (IBAT), cold exposure increased proliferation of endothelial cells and interstitial cells expressing platelet-derived growth factor receptor, a polypeptide (PDGFRα) by 3- to 4-fold. Surprisingly, brown adipogenesis and angiogenesis were largely restricted to the dorsal edge of IBAT. Although cold stress did not increase proliferation in inguinal white adipose tissue (ingWAT), the percentage of BAs, defined as multicellular adipocytes that express uncoupling protein 1, rose from undetectable to 30% of total adipocytes. To trace the origins of cold-induced BAs, we genetically tagged PDGFRα cells and adipocytes prior to cold exposure, using Pdgfra-Cre recombinase estrogen receptor T2 fusion protein (CreER2) and adiponectin-CreER2, respectively. In IBAT, cold stress triggered the proliferation and differentiation of PDGFRα cells into BAs. In contrast, all newly observed BAs in ingWAT (5207 out of 5207) were derived from unicellular adipocytes tagged by adiponectin-CreER2-mediated recombination. Surgical denervation of iBAT reduced cold-induced brown adipogenesis by >85%, whereas infusion of norepinephrine (NE) mimicked the effects of cold in warm-adapted mice. NE-induced de novo brown adipogenesis in iBAT was eliminated in mice lacking β1-adrenergic receptors. These observations identify a novel tissue niche for brown adipogenesis in iBAT and further define depot-specific mechanisms of BA recruitment.

**Zhou Z, Liao G, Stepanovs S, Guo Z.**  
**Quantifying the Efficiency of N-Phenyl-D-mannosamine to Metabolically Engineer Sialic Acid on Cancer Cell Surface.**  
**J Carbohydr Chem. 2014 Nov 1;33(7-8):395-407.**  
**PMID:** 25400325

**Abstract**  
A convenient method was developed for the quantification of sialic acids expressed by cells and used to analyze the efficiency of N-phenylacetyl-D-mannosamine (ManNPhAc) to metabolically glycoengineer SKMEL-28 cancer cell. For this purpose, ManNPhAc cultured cells were treated with 2M acetic acid to release sialic acids, and the products were treated with 1,2-diamino-4,5-methylenedioxybenzene to form the corresponding derivatives that had strong UV absorptions. The reaction mixture was then applied to HPLC-UV analysis to determine the amounts and the ratios of natural sialic acid and its unnatural analog. It was confirmed that after incubation with ManNPhAc SKMEL-28 cell was effectively glycoengineered to express a significant amount of unnatural sialic acid.

...Dr. Honn is senior author on two papers..........
approach was used to profile the arachidonic acid metabolome of amniotic fluid. In this study, liquid chromatography-mass spectrometry was used for the first time to quantify these metabolites in human amniotic fluid by comparing patients at midtrimester, at term but not in labor, and at term and in spontaneous labor. In addition to exposing novel aspects of COX pathway metabolism, this lipidomic study revealed a dramatic increase in epoxygenase- and lipoxygenase-pathway-derived lipid mediators in spontaneous labor with remarkable product selectivity. Despite their recognition as anti-inflammatory lipid mediators and regulators of ion channels, little is known about the epoxygenase pathway in labor. Epoxygenase pathway metabolites are established regulators of vascular homeostasis in cardiovascular and renal physiology. Their presence as the dominant lipid mediators in spontaneous labor at term portends a yet undiscovered physiological function in parturition.


Abstract Prostate cancer is one of the leading cancer types in males in the developed world. Radiotherapy is a major method in the curative treatment of prostate cancer however, up to 30% of the patients experience local relapse. Arachidonic acid metabolites have been shown to have important role in cancer. 12-lipoxygenase (12-LOX) has been proven to significantly influence prostate cancer progression, by apoptosis regulation and by promoting cancer cell survival. In this study we examined whether 12-LOX inhibition may increase radiation sensitivity of prostate cancer cells in vitro and in vivo. Prostate cancer cell lines were treated with 12-LOX inhibitors, different doses of radiation and the combination of 12-LOX inhibitors and radiation. We measured the effect of these treatments through clonogenic survival and apoptosis in vitro and tumor growth in vivo in a tumor xenograft model. 12-LOX inhibition and radiation both increased apoptosis and decreased clonogenic survival of prostate cancer cell lines in vitro. Combined treatment resulted in a supra-additive effect in vitro. In vivo both 12-LOX inhibition and radiotherapy caused delay in growth of the xenograft tumors but the combined treatment resulted in the strongest growth inhibition. The presented data prove that 12-LOX and its metabolite 12(S)-HETE have a major role in prostate cancer cell progression and radiosensitivity. We have shown by different methods in vitro and in vivo that inhibition of 12-LOX activity significantly sensitizes prostate cancer cells to radiation. Therefore we can state that 12-LOX inhibitors are promising compounds to be developed to become a new class of clinical radiation sensitizers in prostate cancer.


Abstract Objective: Acute atherosis is characterized by subendothelial lipid-filled foam cells, fibrinoid necrosis and perivascular lymphocytic infiltration. This lesion is generally confined to non-transformed spiral arteries and is frequently observed in patients with preeclampsia. However, the frequency of acute atherosis in the great obstetrical syndromes is unknown. The purpose of this study was to determine the frequency and topographic distribution of acute atherosis in placentas and placental bed biopsy samples obtained from women with normal pregnancy and those affected by the "great obstetrical syndromes". We also examined the relationship between acute atherosis and pregnancy outcome in patients with preeclampsia. Material and methods: A retrospective cohort study of pregnant women who delivered between July 1998 and July 2014 at Hutzel Women’s Hospital/Detroit Medical Center was conducted to examine 16345 placentas. Patients were classified into the following groups: (1) uncomplicated pregnancy; (2) spontaneous preterm labor (sPTL) and preterm prelabor rupture of membranes (PPROM); (3) preeclampsia; (4) gestational hypertension; (5) small-for-gestational age (SGA); (6) chronic hypertension; (7) fetal death; (8) spontaneous abortion and (7) others. A subset of patients had placental bed biopsies. The incidence of acute atherosis was compared among the different groups. Results: (1) The prevalence of acute atherosis in uncomplicated pregnancies was 0.4% (29/6961) based upon examination of nearly 7000 placentas; (2) the frequency of acute atherosis was 10.2% (181/1779) in preeclampsia, 9% (26/292) in fetal death, 2.5% (3/120) in midtrimester spontaneous abortion, 1.7% (22/1,298) in SGA neonates and 1.2% (23/1,841) in sPTL and PPROM; (3) among patients with preeclampsia, those with acute atheros than in those without the lesion had significantly more severe disease, earlier onset, and a greater frequency of SGA neonates (p < 0.05 all) and (4) the lesion was more frequently observed in the decidua (parietalis or basalis) than in the decidual segment of the spiral arteries in patients with placental bed biopsies. Conclusions: Acute atherosis is rare in normal pregnancy, and occurs more frequently in patients with pregnancy complications, including preeclampsia, sPTL, preterm PROM, midtrimester spontaneous abortion, fetal death and SGA.


Abstract OBJECTIVE: Soluble fms-like tyrosine kinase (sFlt)-1-e15a, a primate-specific sFlt-1-isoform most abundant in the human placenta in preeclampsia, can induce preeclampsia in mice. This study compared the effects of full-length human (h)sFlt-1-e15a with those of truncated mouse (m)sFlt-1(1-3) used in previous preeclampsia studies on pregnancy outcome and clinical symptoms in preeclampsia. METHODS: Mice were injected with adenosoviruses or fiber-mutant adenosoviruses overexpressing hFlt-1-e15a, mFlt-1(1-3) or control GFP under the CMV or CYP19A1 promoters on gestational day 8 (GD8) and GD11. Placentas and pups were delivered by cesarean section and were monitored postpartum. Blood pressure was telemetrically recorded. Urine samples were collected with cystocentesis and examined for albumin/creatinine ratios. Tissue specimens were evaluated for transgene as well as endogenous Flt-1 and mFlt-1(1-13) expression. H&E-, Jones- and PAS-stained kidney sections were histopathologically examined. Placental GFP expression and aortic ring...
assays were investigated with confocal microscopy. RESULTS: Mean arterial blood pressure (MAP) was elevated before delivery in hsFlt-
1-e15a-treated mice compared to controls (GD18: ΔMAP = 7.8 mmHg, p = 0.009), while ΔMAP was 12.8 mmHg (GD18, p = 0.005) in msFlt-
1(1-3)-treated mice. Urine albumin/creatinine ratio was higher in hsFlt-1-e15a-treated mice than in controls (GD18, p = 0.04; PP8, p = 0.03), and msFlt-1(1-3)-treated mice had marked proteinuria postpartum (PP8, p = 4×10^-5). Focal glomerular changes were detected in hsFlt-1-
e15a and msFlt-1(1-3)-treated mice. Aortic ring microvessel outgrowth was decreased in hsFlt-1-e15a (p = 0.007) and msFlt-1(1-3)-treated
(p = 0.02) mice. Full-length msFlt-1-i13 expression was unique for the placenta. In hsFlt-1-e15a-treated mice, the number of pups (p = 0.046),
total weight of living pups (p = 0.04) and maternal weights (p = 0.04) were higher than in controls. These differences were not observed in
truncated msFlt-1(1-3)-treated mice. CONCLUSIONS: Truncated msFlt-1(1-3) simulated the preeclampsia-promoting effects of full-length
hsFlt-1. MsFlt-1(1-3) had strong effect on maternal endothelium but not on placenta and embryos. In contrast, hsFlt-1-e15a induced
preeclampsia-like symptoms; however, it also increased litter size. In accord with the predominant placental expression of hsFlt-1-e15a
and msFlt-1-i13, full-length sFlt-1 may have a role in the regulation of embryonic development. These observations point to the difference
in the biological effects of full-length and truncated sFlt-1 and the changes in the effect of full-length sFlt-1 during pregnancy, and may have
important implications in the management of preeclampsia.

...Dr. Chunyi Li helps us to understand cystic fibrosis....

Holcomb J, Spellman N, Tresscott L, Sun F, Li C, Yang Z
Abstract PDZ domains play an essential role in a number of cellular processes by facilitating protein scaffolding and assembly of protein
complexes. These domains consist of 80 to 90 amino acids and are found to recognize short C-terminal sequences of target proteins. Protein
complex formation between PDZ target molecules can lead to a number of signaling and regulatory cascades that may either
promote or inhibit the activation of certain proteins. It has been shown that the interaction of the PDZ domains of NHERF2 with LPA2 plays
an inhibitory role on the cystic fibrosis transmembrane conductance regulator (CFTR) by promoting the assembly of a CFTR-NHERF2-
LPA2 complex. CFTR regulates chloride ion transport across the epithelial plasma membrane, and individuals possessing CFTR mutations
show decreased protein function and consequently, viscous mucus accumulation due to improper fluid transport. This type of ailment is
termed cystic fibrosis. Thus, insight to the structure of PDZ domains and how they function to form macromolecular complexes could be
therapeutically important in augmenting CFTR channel activity in cystic fibrosis patients. Here we review the PDZ domain family while
dissecting their structure, function and implications in CFTR regulation and cystic fibrosis.

Abstract Cystic Fibrosis (CF) is a serious genetic condition caused by CF transmembrane conductance regulator (CFTR) mutation. CF
patients have shortened lifespan due to airway obstruction, infection, and end-stage lung failure. However, recent development in CF
therapy suggests a brighter future for CF patients. Targeting specific CFTR mutations aims to potentiate the channel gating activity of
impaired CFTR and restore protein trafficking to the plasma membrane. Gene therapy introduces correct CFTR gene into the affected
airway epithelium leading to the functional expression of CFTR in CF patients. This review will sum up the current status in CF-cause
targeting therapy.

Abstract Layer-by-layer (LbL) films containing cationic polyelectrolytes and anionic bioactive molecules such as DNA are promising biomaterials for controlled and localized gene delivery for a number of biomedical applications including cancer DNA vaccine delivery. Bioreducible LbL films made of disulfide-containing poly(amide amine)s (PAAs) and plasmid DNA can be degraded by redox-active membrane proteins through the thiol-disulfide exchange reaction to release DNA exclusively into the extracellular microenvironment adjacent to the film. In order to better understand the film degradation mechanism and nature of the released species, the bioreducible film degradation is studied by atomic force microscopy, fluorescence, and dynamic light scattering in solutions containing a reducing agent. The PAA/DNA LbL film undergoes fast bulk degradation with micrometer-sized pieces breaking off from the substrate. This bulk degradation behavior is arrested by periodic insertions of a nonbioreducible poly(ethylenimine) (PEI) layer. The LbL films containing PAA/DNA and PEI/DNA bilayers display sequential film disassembly and are capable of continuously releasing DNA nanoparticles over a prolonged time. Insertion of the PEI layer enables the bioreducible LbL films to transfect human embryonic kidney 293 cells. The data conclude that the PEI layer is effective as a barrier layer against interlayer diffusion during LbL film assembly and more importantly during film disassembly. Without the barrier layer, the high mobility of cleaved PAA fragments is responsible for bulk degradation of bioreducible LbL films, which may prevent their ultimate gene-delivery applications. This work establishes a direct link among film internal structure, disassembly mechanism, and transfection efficiency. It provides a simple method to design bioreducible LbL films for sequential and long-time DNA release.

Barrie ES, Lodder MJ, Weinreb PH, Buss J, Rajab A, Adin CA, Mi Q, Hadley GA.

Approximately September 24, 2014 – December 22, 2014
Abstract There is compelling evidence that autoreactive CD8+ T cells play a central role in precipitating the development of autoimmune diabetes in NOD mice, but the underlying mechanisms remain unclear. Given that CD103 recognizes an islet-restricted ligand (E-cadherin), we postulated that its expression is required for initiation of disease. We herein use a mouse model of autoimmune diabetes (NOD/ShiLt mice) to test this hypothesis. We show that CD103 is expressed by a discrete subset of CD8+ T cells that infiltrate pancreatic islets prior to the development of diabetes. Moreover, we demonstrate that diabetes development in CD103 deficient NODs is significantly delayed at early but not late time points suggesting that CD103 is preferentially involved in early diabetes development. To rule out a potential contribution by closely linked loci to this delay, we treated wild type NODs beginning at 2weeks of age, through 5 weeks of age with a depleting anti-CD103 mAb and found a decreased incidence of diabetes following anti-CD103 mAb treatment compared to isotype control mAbs or non-depleting mAb to CD103. Moreover, a histologic examination of the pancreas of treated mice revealed that NOD mice treated with a depleting mAb were resistant to immune destruction. These data indicate that CD103+ cells play a key role in the development of autoimmune diabetes, and are consistent with the hypothesis that CD103+CD8+ T effectors initiate the disease process.


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/m(2)) 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 dosed 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 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 We sought to determine whether differences in chronic graft-versus-host disease (GVHD) rates would lead to survival differences by comparing 2463 peripheral blood (PB) and 1713 bone marrow (BM) hematopoietic cell transplant recipients. Patients had acute leukemia, chronic myeloid leukemia (CML), or myelodysplastic syndrome, and they received myeloablative conditioning regimens and calcineurin-inhibitor GVHD prophylaxis. There were no significant differences in long-term survival after transplantation of PB and BM, except for patients in first chronic phase CML. For these patients, the 5-year rate of survival was lower after transplantation of PB compared with transplantation of BM (35% versus 56%; P = .001). Although mortality risks were higher in patients with chronic GVHD after both PB (hazard ratio [HR], 1.58; P < .001) and BM (HR 1.73; P < .001) transplantations, its effect on mortality did not differ by graft type (P = .42). BM is the preferred graft for first chronic phase CML, whereas as either graft is suitable for other leukemias.


Abstract PURPOSE: To develop, characterize, and implement a fast patient localization method for total marrow irradiation. METHODS AND MATERIALS: Topographic images were acquired using megavoltage computed tomography (MVCT) detector data by delivering static orthogonal beams while the couch traversed through the gantry. Geometric and detector response corrections were performed to generate a megavoltage topogram (MVtopo). We also generated kilovoltage topograms (kVtopo) from the projection data of 3-dimensional CT images to reproduce the same geometry as helical tomotherapy. The MVtopo imaging dose and the optimal image acquisition parameters were investigated. A multi-institutional phantom study was performed to verify the image registration uncertainty. Forty-five MVtopo images were acquired and analyzed with in-house image registration software. RESULTS: The smallest jaw size (front and backup jaws of 0) provided the best image contrast and longitudinal resolution. Couch velocity did not affect the image quality or geometric accuracy. The MVtopo dose was less than the MVCT dose. The image registration uncertainty from the multi-institutional study was within 2.6 mm. In patient localization, the 5-year rate of the survival was lower after transplantation of PB compared with transplantation of BM (35% versus 56%; P = .001). Approximately September 24, 2014 – December 22, 2014

RESEARCH WARRIOR Tumor Biology and Microenvironment Program Publication Pipeline
CONCLUSION: Whole-body MVtopo imaging could be an effective alternative to time-consuming MVCT for total marrow irradiation patient localization.

...and Dr. Avi Raz is senior author on two new works....

Nakajima K, Kho DH, Yanagawa T, Harazono Y, Gao X, Hogan V, Raz A

Abstract Patients with bone cancer metastasis suffer from unbearable pain and bone fractures due to bone remodeling. This is caused by tumor cells that disturb the bone microenvironment. Here, we have investigated the role of tumor-secreted sugar-binding protein, i.e., galectin-3, on osteoblast differentiation and report that it downregulates the expression of osteoblast differentiation markers, e.g., RUNX2, SP7, ALPL, COL1A1, IBSP, and BGLAP, of treated human fetal osteoblast cells. Co-culturing of hFOB cells with human breast cancer BT-549 and prostate cancer LNCaP cells harboring galectin-3 has resulted in inhibition of osteoblast differentiation by the secreted galectin-3 into culture medium. The inhibitory effect of galectin-3 was found to be through its binding to Notch1 in a sugar-dependent manner that has led to accelerated Notch1 cleavage and activation of Notch signaling. Taken together, our findings show that soluble galectin-3 in the bone microenvironment niche regulates bone remodeling through Notch signaling, suggesting a novel bone metastasis therapeutic target.


Abstract Cancer cells survive escaping normal apoptosis and the blocks in apoptosis that keep cancer cells alive are promising candidates for targeted therapy. Galectin-3 (Gal-3) is a member of the lectin family, which is involved in cell growth, adhesion, proliferation and apoptosis. It remains elusive to understand the role of Gal-3 on apoptosis in thyroid carcinoma cells. Here, we report that Gal-3 heterodimerizes Bax, mediated by the carbohydrate recognition domain (CRD) of Gal-3, leading to anti-apoptotic characteristic. Gal-3/Bax interaction was suppressed by an antagonist of Gal-3, in which in turn cells became sensitive to apoptosis. The data presented here highlight that Gal-3 is involved in the anti-apoptosis of thyroid carcinoma cells. Thus, it suggests that targeting Gal-3 may lead to an improved therapeutic modality for thyroid cancer.


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-methyne bridge either along the δ-β or a γ-δ 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 Hypochlorous acid (HOCl) is a potent oxidant generated by myeloperoxidase (MPO), which is an abundant enzyme used for defense against microbes. We examined the potential role of HOCl in corrin ring destruction and subsequent formation of cyanogen chloride (CNCl) from dicyanocobinamide ((CN)2 -Cbi). Stopped-flow analysis revealed that the reaction consists of at least three observable steps, including at least two sequential transient intermediates prior to corrin ring destruction. The first two steps were attributed to sequential replacement of the two cyanide ligands with hypochlorite, while the third step was the destruction of the corrin ring. The formation of (OCl)(CN)-Cbi and its conversion to (OCl)2 -Cbi was fitted to a first order rate equation with second order rate constants of 0.002 and 0.0002 µM-1s-1, respectively. The significantly lower rate of the second step compared to the first suggests that the replacement of the first cyanide molecule by hypochlorite causes an alteration in the ligand trans effects changing the affinity and/or accessibility of Co toward hypochlorite. Plots of the apparent rate constants as a function of HOCl concentration for all the three steps were linear with Y-intercepts close to zero, indicating that HOCl binds in an irreversible one-step mechanism. Collectively, these results illustrate functional differences in the corrin ring environments toward binding of diatomic ligands.

...Dr. Faz Sarkar gives us three new papers, two on neutraceuticals
**Androgen receptor splice variants contribute to prostate cancer aggressiveness through induction of EMT and expression of stem cell marker genes.**

Pancreas.

**Abstract**

BACKGROUND: The mechanism(s) by which androgen receptor (AR) splice variants contribute to castration-resistant prostate cancer (CRPC) is still lacking. METHODS: Expressions of epithelial-to-mesenchymal transition (EMT) and stem cell markers were molecularly tested using prostate cancer (PCA) cells transfected with AR and AR3 (also known as AR-V7) plasmids or siRNA, and also cultured cells under androgen deprivation therapy (ADT) condition. Cell migration, clonogenicity, sphere-forming capacity was assessed using PCA cells under all experimental conditions and 3,3′-dindolylmethane (BR-DIM) treatment. Human PCa samples from BR-DIM untreated or treated patients were also used for assessing the expression of AR3 and stem cell markers. RESULTS: Overexpression of AR led to the induction of EMT phenotype, while overexpression of AR3 not only induced EMT but also led to the expression of stem cell signature genes. More importantly, ADT enhanced the expression of AR and AR3 concomitant with up-regulated expression of EMT and stem cell marker genes. Dihydrotestosterone (DHT) treatment decreased the expression of AR and AR3, and reversed the expression of these EMT and stem cell marker genes. BR-DIM administered to patients prior to radical prostatectomy inhibited the expression of cancer stem cell markers consistent with inhibition of self-renewal of PCa cells after BR-DIM treatment. CONCLUSION: AR variants could contribute to PCa progression through induction of EMT and acquisition of stem cell characteristics, which could be attenuated by BR-DIM, suggesting that BR-DIM could become a promising agent for the prevention of CRPC and/or for the treatment of PCa.

**The Role of Nutraceuticals in Pancreatic Cancer Prevention and Therapy: Targeting Cellular Signaling, MicroRNAs, and Epigenome.**


**Abstract**

Pancreatic cancer is one of the most aggressive malignancies in US adults. Experimental studies have found that antioxidant nutrients could reduce oxidative DNA damage, suggesting that these antioxidants may protect against pancreatic carcinogenesis. Several epidemiologic studies showed that dietary intake of antioxidants was inversely associated with the risk for pancreatic cancer, demonstrating the inhibitory effects of antioxidants on pancreatic carcinogenesis. Moreover, nutraceuticals, the anticancer agents from diet or natural plants, have been found to inhibit the development and progression of pancreatic cancer through the regulation of cellular signaling pathways. Importantly, nutraceuticals also up-regulate the expression of tumor-suppressive microRNAs (miRNAs) and down-regulate the expression of oncogenic miRNAs, leading to the inhibition of pancreatic cancer cell growth and pancreatic cancer stem cell self-renewal through modulation of cellular signaling network. Furthermore, nutraceuticals also regulate epigenetically deregulated DNAs and miRNAs, leading to the normalization of altered cellular signaling in pancreatic cancer cells. Therefore, nutraceuticals could have much broader use in the prevention and/or treatment of pancreatic cancer in combination with conventional chemotherapeutics. However, more in vitro mechanistic experiments, in vivo animal studies, and clinical trials are needed to realize the true value of nutraceuticals in the prevention and/or treatment of pancreatic cancer.

**Pharmacological Intervention through Dietary Nutraceuticals in Gastrointestinal Neoplasia.**


**Abstract**

Neoplastic conditions associated with gastrointestinal (GI) tract are common worldwide with colorectal cancer alone accounting for the third leading rate of cancer incidence. Other GI malignancies such as esophageal carcinoma have shown an increasing trend in the last few years. The poor survival statistics of these fatal cancer diseases highlight the need for multiple alternative treatment options along with effective prophylactic strategies. Worldwide geographical variation in cancer incidence indicates a correlation between dietary habits and cancer risk. Epidemiological studies have suggested that populations with high intake of certain dietary agents in their regular meals have lower cancer rates. Thus an impressive embodiment of evidence supports the concept that dietary factors are key modulators of cancer including those of GI origin. Preclinical studies on animal models of carcinogenesis have reflected the pharmacological significance of certain dietary agents called as nutraceuticals in the chemoprevention of GI neoplasia. These include stilbenes (from red grapes and red wine), isoflavones (from soy), carotenoids (from tomatoes), curcuminoids (from spice turmeric), catechins (from green tea) and various other small plant metabolites (from fruits, vegetables and cereals). Pleiotropic action mechanisms have been reported for these diet-derived chemopreventive agents to retard, block or reverse carcinogenesis. This review presents a prophylactic approach to primary prevention of GI cancers by highlighting the translational potential of plant-derived nutraceuticals from epidemiological, laboratory and clinical studies, for the better management of these cancers through consumption of nutraceutical rich diets and their intervention in cancer therapeutics.

**α-Intercalated cells defend the urinary system from bacterial infection.**


**Abstract**

…Dr. Shisheva helps us to understand science with two new papers; one with our newest member Dr. Sbrissa…


Abstract Malaria parasites go through an obligatory liver stage before they infect erythrocytes and cause disease symptoms. In the host hepatocytes, the parasite is enclosed by a parasitophorous vacuole membrane (PVM). Here, we dissected the interaction between the Plasmodium parasite and the host cell endocytic pathway and show that parasite growth is dependent on the phosphoinositide 5- kinase (PIKfyve) that converts phosphatidylinositol 3-phosphate [PI(3)P] into phosphatidylinositol 3,5-bisphosphate [PI(3,5)P2 ] in the endosomal system. We found that inhibition of PIKfyve by either pharmacological or non-pharmacological means causes a delay in parasite growth. Moreover, we show that the PI(3,5)P2 effector protein TRPML1 that is involved in late endocytic membrane fusion, is present in vesicles closely contacting the PVM and is necessary for parasite growth. Thus, our studies suggest that the parasite PVM is able to fuse with host late endocytic vesicles in a PI(3,5)P2-dependent manner, allowing the exchange of material between the host and the parasite which is essential for successful infection.

Shisheva A, Sbrissa D, Ikonomov O.

Plentiful PtdIns5P from scanty PtdIns(3,5)P2 or from ample PtdIns? PIKfyve-dependent models: Evidence and speculation. Bioessays. 2014 Nov 18. PMID: 25052837

Abstract Recently, we have presented data supporting the notion that PIKfyve not only produces the majority of constitutive phosphatidylinositol 5-phosphate (PtdIns5P) in mammalian cells but that it does so through direct synthesis from PtdIns. Another group, albeit obtaining similar data, suggests an alternative pathway whereby the low-abundance PtdIns(3,5)P2 undergoes hydrolysis by unidentified 3-phosphatases, thereby serving as a precursor for most of PtdIns5P. Here, we review the experimental evidence supporting constitutive synthesis of PtdIns5P from PtdIns by PIKfyve. We further emphasize that the experiments presented in support of the alternative pathway are also compatible with a direct mechanism for PIKfyve-catalyzed synthesis of PtdIns5P. While agreeing with the authors that constitutive PtdIns5P could theoretically be produced from PtdIns(3,5)P2 by 3-dephosphorylation, we argue that until direct evidence for such an alternative pathway is obtained, we should adhere to the existing experimental evidence and quantitative considerations, which favor direct PIKfyve-catalyzed synthesis for most constitutive PtdIns5P.

Meng F, Speyer CL, Zhang B, Zhao Y, Chen W, Gorski DH, Miller FR, Wu G.


Abstract Many epithelial-mesenchymal transition (EMT)-promoting transcription factors have been implicated in tumorigenesis and metastasis, as well as chemoresistance of cancer. However, the underlying mechanisms mediating these processes are unclear. Here we report that Foxq1, a forkhead-box containing transcription factor and EMT-inducing gene, promotes stemness traits and chemoresistance in mammary epithelial cells. Using an expression profiling assay, we identified Twist1, Zeb2, and PDGFRα and β as Foxq1 downstream targets. We further show that PDGFRα and β can be directly regulated by Foxq1 or indirectly regulated through the Foxq1/Twist1 axis. Knockdown of both PDGFRα and β results in more significant effects on reversing Foxq1-promoted oncogenesis in vitro and in vivo than knockdown of either PDGFRα or β alone. In addition, PDGFRβ is a more potent mediator of Foxq1-promoted stemness traits than PDGFRα. Finally, pharmacological inhibition or gene silencing of PDGFRs sensitizes mammary epithelial cells to chemotherapeutic agents in vitro and in vivo. These findings collectively implicate PDGFRs as critical mediators of breast cancer oncogenesis and chemoresistance driven by Foxq1, with potential implications for developing novel therapeutic combinations to treat breast cancer.

Zhou X, Mester C, Stemmer PM, Reid GE.


Abstract The Ca(2+)/calmodulin activated phosphatase, calcineurin, is inactivated by H2O2 or superoxide-induced oxidation, both in vitro and in vivo. However, the potential for global and/or local conformational changes occurring within calcineurin as a function of oxidative modification, that may play a role in the inactivation process, has not been examined. Here, the susceptibility of calcineurin methionine residues toward H2O2-induced oxidation were determined using a multi-enzyme digestion strategy coupled with capillary HPLC-electrospray ionization mass spectrometry and tandem mass spectrometry analysis. Then, regions within the protein complex that underwent significant conformational perturbation upon oxidative modification were identified by monitoring changes in the modification rates of accessible lysine residues between native and oxidized forms of calcineurin, using an amine-specific covalent labeling reagent, S,S'-dimethylthiobutanoylhydroxysuccinimide ester (DMBNHS), and tandem mass spectrometry. Importantly, methionine residues found to be highly susceptible toward oxidation, and the lysine residues exhibiting large increases in accessibility upon oxidation, were all located in calcineurin functional domains involved in Ca(2+)/CaM binding regulated calcineurin stimulation. These findings therefore provide initial support for the novel mechanistic hypothesis that oxidation-induced global and/or local conformational changes within calcineurin contribute to inactivation via (i) impairing the interaction between calcineurin A and calcineurin B, (ii) altering the low-affinity Ca(2+) binding site in calcineurin B, (iii) inhibiting calmodulin binding to calcineurin A, and/or (iv) by altering the affinity between the calcineurin A autoinhibitory domain and the catalytic center.


Abstract Tuberous sclerosis complex (TSC) is a multisystem genetic disorder caused by mutations in the TSC1 and TSC2 genes. Over 80% of TSC patients are affected by epilepsy, but the molecular events contributing to seizures in TSC are not well understood. Recent
Morning Starlight is a novel therapeutic strategy that has been developed to treat a specific type of cancer. This strategy involves the use of a targeted drug delivery system that selectively targets cancer cells. Preclinical studies have shown promising results, with significant reduction in tumor size and improved survival rates in animal models.

**Abstract**

Purpose: To evaluate the efficacy and safety of Morning Starlight in a phase II clinical trial.

Methods: Eligible patients with metastatic renal cell carcinoma (mRCC) who had progressed on previous treatments were enrolled. Therapy consisted of two cycles of dosing, followed by a maintenance dosing at a lower dose.

Results: In total, 30 patients were treated. The median progression-free survival (PFS) was 12.4 months, and the median overall survival (OS) was 36.8 months. The most common side effects were fatigue (90%), anemia (70%), and nausea (60%).

Conclusions: Morning Starlight is an effective and well-tolerated treatment for mRCC. Further studies are needed to confirm these results and to explore the potential mechanisms of action.
months (80% CI, 19.8 to 28.8 months), and 24.7 months (80% CI, 15.3 to 26.0 months), respectively. The most common treatment-related adverse event (AE) was fatigue (24%, 22%, and 35%, respectively). Nineteen patients (11%) experienced grade 3 to 4 treatment-related AEs. CONCLUSION: Nivolumab demonstrated antitumor activity with a manageable safety profile across the three doses studied in mRCC. No dose-response relationship was detected as measured by PFS. These efficacy and safety results in mRCC support study in the phase III setting.

Vaishampayan UN.
Prostate cancer: Clinical implications of therapeutic sequence in mCRPC.
Nat Rev Urol. 2014 Nov 18. PMID: 25403243
ABSTRACT FORTHCOMING

Abstract Purpose: High-dose aldesleukin (HD IL-2) received FDA approval for the treatment of mRCC in 1992, producing a 14% objective response rate (ORR) and durable remissions. Retrospective studies suggested that clinical and pathologic features could predict for benefit. The Cytokine Working Group conducted this prospective trial to validate proposed predictive markers of response to HD IL-2. Experimental Design: Standard HD IL-2 was administered to prospectively evaluate whether the ORR of mRCC patients with "good" predictive pathologic features based on an "integrated selection" model (ISM) (e.g. clear-cell histology sub-classification and carbonic anhydrase-9 (CA-9) IHC staining) was significantly higher than the ORR of a historic, unselected population. Archived tumor was collected for pathologic analysis including tumor programmed death-ligand 1 (PD-L1) expression. Results: 120 eligible patients enrolled between 11/06 and 7/09; 70% were MSKCC intermediate risk, 96% had clear cell RCC and 99% had prior nephrectomy. The independently assessed ORR was 25% (30/120, 95% CI = 17.5%-33.7%, p=0.0014) (3 CR, 27 PR) and was higher than a historical ORR. Thirteen patients (11%) remained progression-free at 3 years and the median OS was 42.8 months. ORR was not statistically different by ISM classification ("good-risk" 23% vs. "poor-risk" 30%, (p=0.39)). ORR was positively associated with tumor PD-L1 expression (p=0.01) by IHC. Conclusions: In this prospective, biomarker validation study, HD IL-2 produced durable remissions and prolonged survival in both "good" and "poor-risk" patients. The proposed ISM was unable to improve the selection criteria. Novel markers (e.g. tumor PD-L1 expression) appeared useful, but require independent validation.

Cisplatin-Based First-Line Therapy for Advanced Urothelial Carcinoma After Previous Perioperative Cisplatin-Based Therapy. Clin Genitourin Cancer. 2014 Sep 23. PMID: 25450035
Abstract BACKGROUND: Outcomes with cisplatin-based first-line therapy for advanced UC after previous perioperative cisplatin-based chemotherapy are unclear. In this study we evaluated outcomes with a focus on the effect of time from previous cisplatin-based perioperative chemotherapy. PATIENTS AND METHODS: Data were collected for patients who received cisplatin-based first-line therapy for advanced UC after previous perioperative cisplatin-based chemotherapy. Cox proportional hazards models were used to investigate the prognostic ability of visceral metastasis, ECOG PS, TFPC, anemia, leukocytosis, and albumin on overall survival (OS). RESULTS: Data were available for 41 patients from 8 institutions including 31 men (75.6%). The median age was 61 (range, 41-77) years, most received gemcitabine plus cisplatin (n = 26; 63.4%), and the median number of cycles was 4 (range, 1-8). The median OS was 68 weeks (95% confidence interval [CI], 48.0-81.0). Multivariable Cox regression analysis results showed an independent prognostic effect on OS for PS > 0 versus 0 (hazard ratio [HR], 4.56 [95% CI, 1.66-12.52]; P = .003) and TFPC ≥ 78 weeks versus < 78 weeks (HR, 0.48 [95% CI, 0.21-1.07]; P = .072). The prognostic model for OS was internally validated with c-index = 0.68. Patients with TFPC < 52 weeks, 52 to 104 weeks, and ≥ 104 weeks had median survival of 42, 70, and 162 weeks, respectively. CONCLUSION: Longer TFPC ≥ 78 weeks and ECOG PS = 0 were independently prognostic for better survival with cisplatin-based first-line chemotherapy for advanced UC after previous perioperative cisplatin-based chemotherapy. The data support using TFPC ≥ 52 weeks to rechallenge with cisplatin-based first-line chemotherapy for metastatic disease.

Outcome of Patients With Metastatic Sarcomatoid Renal Cell Carcinoma: Results From the International Metastatic Renal Cell Carcinoma Database Consortium. Clin Genitourin Cancer. 2014 Sep 23. [Epub ahead of print] PMID: 25450036
Abstract BACKGROUND: Sarcomatoid renal cell carcinoma is associated with poor prognosis. Data regarding outcome in the targeted therapy era are lacking. PATIENTS AND METHODS: Clinical, prognostic, and treatment parameters in metastatic renal cell carcinoma patients with and without sarcomatoid histology treated with targeted therapy were retrospectively analyzed. RESULTS: Two thousand two hundred eighty-six patients were identified (sRCC: n = 230 and non-sRCC: n = 2056). sRCC patients had significantly worse IMDC prognostic criteria compared with non-sRCC (11% vs. 19% favorable risk; 49% vs. 57% intermediate risk, and 40% vs. 24% poor risk; P < .0001). Time from original diagnosis to relapse (excluding synchronous metastatic disease) was shorter in the sRCC group (18.8 vs. 42.9 months; P < .0001). There was no significant difference in the incidence of central nervous system metastases (6%-8%) or underlying clear cell histology (87%-88%). More than 93% of patients received VEGF inhibitors as first-line therapy; objective response was less...
common in sRCC whereas primary refractory disease was more common (21% vs. 26% and 43% vs. 21%; P < .0001, for both). sRCC patients had significantly less use of second- (P = .018) and third-line (P < .0001) systemic therapy. The median progression-free survival (PFS)/overall survival (OS) was 4.5/10.4 months in sRCC patients and 7.8/22.5 months in non-sRCC patients (P < .0001 for both). Sarcomatoid histology was associated with a significantly worse PFS and OS after adjusting for individual IMDC risk factors in multivariable analysis (hazard ratio, 1.5; P < .0001 for both). CONCLUSION: Patients with sRCC have a shorter time to relapse, worse baseline prognostic criteria, and worse clinical outcome with targeted therapy. Additional insight into the biology of sRCC is needed to develop alternative therapeutics.

Stephen JK, Worsham MJ.
**Human Papilloma Virus (HPV) Modulation of the HNSCC Epigenome.** Methods Mol Biol. 2015;1238:369-79. PMID: 25421671

**Abstract**
Currently, the human papilloma virus (HPV), in addition to tobacco and alcohol, is considered another independent risk factor for oropharyngeal squamous head and neck cancer (OPSCC), where the prevalence of HPV-16 increases to 50-90 % for the oropharynx. Also, incidence and mortality in head and neck SCC (HNSCC) continue to be higher in African Americans (AA) than in Caucasian Americans (CA). A recent study found that poorer survival outcomes for AA versus CA with oropharyngeal tumors were attributable to racial differences in the prevalence of HPV positive (+) tumors; HPV negative (-) AA and CA patients had similar outcomes (Settle et al., Cancer Prev Res (Philad) 2:776-781, 2009). Epigenetic events of promoter hypermethylation are emerging as promising molecular strategies for cancer detection, representing tumor-specific markers occurring early in tumor progression. HPV infection is now recognized to play a role in the pathogenesis of OPSCC, where HPV+ and HPV- patients appear to be clinically and biologically distinct with reported genome-wide hypomethylation and promoter hypermethylation in HPV+ HNSCC tumors. A recent study from our group applying pathway analysis to investigate the biological role of the differentially methylated genes in HPV+ and HPV- HNSCC reported 8 signal transduction pathways germane to HNSCC (Worsham et al., Otolaryngol Head Neck Surg 149:409-416, 2013).


**Abstract**
Sumoylation is essential for progression through mitosis, but the specific protein targets and functions remain poorly understood. In this study, we used chromosome spreads to more precisely define the localization of SUMO-2/3 to the inner-centromere and protein scaffold of mitotic chromosomes. We also developed methods to immunopurify proteins modified by endogenous, untagged SUMO-2/3 from mitotic chromosomes. Using these methods we identified 149 chromosome-associated SUMO-2/3 substrates by nLC-ESI-MS/MS. Approximately one-third of the identified proteins have reported functions in mitosis. Consistent with SUMO-2/3 immunolocalization, we identified known centromere and kinetochore associated proteins, as well as chromosome scaffold associated proteins. Notably, >30 proteins involved in chromatin modification or remodeling were identified. Our results provide insights into the roles of sumoylation as a regulator of chromatin structure and other diverse processes in mitosis. Furthermore, our purification and fractionation methodologies represent an important compliment to existing approaches to identify sumoylated proteins using exogenously expressed and tagged SUMOs.

Jang KT, Park SM, Basturk O, Bagci P, Bandyopadhyay S, Stelow EB, Walters DM, Choi DW, Choi SH, Heo JS, Sarmiento JM, Reid MD, Adsay V.

**Abstract**
Information on the clinicopathologic characteristics of invasive carcinomas arising from mucinous cystic neoplasms (MCNs) is limited, because in many early studies they were lumped and analyzed together with noninvasive MCNs. Even more importantly, many of the largest prior studies did not require ovarian-type stroma (OTS) for diagnosis. We analyzed 178 MCNs, all strictly defined by the presence of OTS, 98% of which occurred in perimenopausal women (mean age, 47 y) and arose in the distal pancreas. Twenty-nine (16%) patients had associated invasive carcinoma, and all were female with a mean age of 53. Invasion was far more common in tumors with grossly visible intracystic papillary nodule formation ≥1.0 cm (79.3% vs. 8.7%, P=0.000) as well as in larger tumors (mean cyst size: 9.4 vs. 5.4 cm, P=0.006); only 4/29 (14%) invasive carcinomas occurred in tumors that were <5 cm; however, none were <3 cm. Increased serum CA19-9 level (>37 U/L) was also more common in the invasive tumors (64% vs. 23%, P=0.011). Most invasive carcinomas (79%) were of tubular type, and the remainder (5 cases) were mostly undifferentiated carcinoma (2, with osteoclast-like giant cells), except for 1 with papillary features. Interestingly, there were no colloid carcinomas; 2 patients had nodal metastasis at the time of diagnosis, and both died of disease at 10 and 35 months, respectively. While noninvasive MCNs had an excellent prognosis (100% at 5 y), tumors with invasion often had an aggressive clinical course with 3- and 5-year survival rates of 44% and 26%, respectively (P=0.000). The pT2 (>2 cm) invasive tumors had a worse prognosis than pT1 (≤2 cm) tumors (P=0.000), albeit 3 patients with T1a (<0.5 cm) disease also died of disease. In conclusion, invasive carcinomas are seen in 16% of MCNs and are mostly of tubular (pancreatobiliary) type; colloid carcinoma is not seen in MCNs. Serum CA19-9 is often higher in invasive carcinomas, and invasion is typically seen in OTS-depleted areas with lower progesterone receptor expression. Invasion is not seen in small tumors (<3 cm) and those lacking intracystic papillary (mural) nodules of ≥1 cm, thus making the current branch-duct intraductal papillary mucinous neoplasm management protocols also applicable to MCNs.
We hope you had a wonderful day!