Cedars-Sinai Research Day V

January 17, 2014

ABSTRACTS

Behavioral Science

Risk factors for recurrent high grade vaginal intraepithelial neoplasia and progression to carcinoma

Joshua Cohen, Sukrant Mehta, BJ Rimel, Christine Walsh, Andrew Li, Beth Karlan, Ilana Cass

Objectives: High grade vaginal intraepithelial neoplasia (VAIN) II-III has a variable clinical course, and due to its relative rarity, data on the efficacy of treatment and risk of recurrence and progression to carcinoma is limited. Our objective was to evaluate predictors of persistence of disease and describe the risk of progression to carcinoma in a cohort of women with high grade VAIN. Methods: Under an IRB-approved protocol we retrospectively identified 44 patients with biopsy proven VAIN II-III from 1995 to 2013; 11 patients with VAIN II and 33 patients with VAIN III were identified who had adequate follow-up . Demographics, treatment, and clinical course were documented. Patients were followed with regular semi-annual colposcopy and biopsies at the discretion of the attending gynecologic oncology physician. Standard statistical analyses were applied. Results: Median age of the cohort is 59 years old (range 20 - 86). Median follow up is 39 months. 34 patients (75%) had a prior diagnosis of cervical intraepithelial neoplasia (CIN), 16 patients (36%) had a prior hysterectomy due to early stage cervical cancer (n=5) or CIN III (n=11). 13 patients (30%) had a prior hysterectomy for benign indications. 4 patients (36%) with VAIN II and 14 patients (42%) with VAIN III recurred over treatment follow-up. Primary treatment with topical therapy, CO2 laser, and surgical excision, had similar rates of recurrence 50%, 40%, and 27% respectively. There were no statistically significant risk factors associated with recurrence. 4 patients (9%) progressed to invasive vaginal or vulvar cancer while 2 patients (5%) developed ano-rectal cancer during surveillance. Median time to progression to cancer was 64 months (range 30 to 101). There was no difference in median age at time of initial dysplasia diagnosis between those who progressed to malignancy and the remainder of patients. Conclusions: In this small cohort, patients with high grade VAIN had a high risk of recurrence irrespective of type of treatment. There were no clear predictors of recurrence based on traditional risk factors for lower genital tract dysplasia or histopathologic criteria. VAIN can progress to invasive cancer of the lower genital tract, suggesting the need for ongoing evaluation with cytology and comprehensive colposcopy by a skilled specialist.

Too much, too late: aggressive measures and the timing of end-of life care discussions in women with gyncologic malignancies

Ilana Cass, Mae Zakhour, Lia Labrant, BJ Rimel, Andrew Li, Christine Walsh, Beth Karlan

OBJECTIVES: To describe the timing of end of life (EOL) discussions and the use of aggressive measures in women who died of progressive gynecologic malignancies at a single institution. METHODS: An IRB-approved retrospective chart review identified 136 patients who died of gynecologic cancer between 2010-2012 with > 1 documented interaction with their treating oncologist in the last 6 months of life. Aggressive measures were defined as chemotherapy within the last 14 days of life, ER visits, hospital and

ICU admissions within the last 30 days of life and inpatient deaths. Utilization of hospice care and how often and where EOL conversations occurred as documented in the medical record from inpatient and outpatient encounters was recorded. RESULTS: Ninety-seven (71%) patients had a documented EOL conversation. Eighteen (19%) of these patients had this discussion as an outpatient at a median of 22 days before death. Of the 79 who had an EOL conversation while inpatient, 27 (34%) died in hospital with a median time to death of 9 days. Two additional patients died in hospital without any documented EOL discussion. Thirteen patients (10%) had chemotherapy in the last 14 days of life. In the last 30 days of life: 54 (40%) were evaluated in the ER, 66 (49%) were admitted into hospital (median 11 day LOS) and 16 (12%) were admitted to the ICU (median 8 day LOS). At the time of death, 55 (40%) patients were enrolled in outpatient hospice care. The mean amount of time in hospice was 28 days; however half the patients initiated hospice within the last 14 days of life. CONCLUSIONS: End of life care discussions rarely occurred in the outpatient setting, consequently an inpatient encounter became the trigger for a discussion of hospice and code status. Evaluation in the ER frequently resulted in escalation of care. Earlier EOL care discussions resulted in less aggressive measures. These data highlight the need for earlier end of life care discussions for women with progressive gynecologic cancers. Table 1. Utilization of aggressive care and hospice care stratified by timing of end-of-life discussion EOL discussion >30 days before death (n=33)No EOL discussion >30 days before death (n=103)PER visit last 30 days9 (27%)45 (44%)0.1Inpatient last 30 days9 (27%)57 (55%)0.005Chemo last 14 days0 (0%)13 (13%)0.04Died in hospital4 (12%)25 (24%)0.2Received hospice care21 (64%)34 (33%)0.002

Breast Cancer following Ovarian Cancer in BRCA mutation carriers: What is the Cost of Surveillance?

Daniel Paik, Farin Amersi, Bresee Catherine, Catherine Dang, Beth Karlan, Andrew Li, Christine Walsh, BJ Rimel, Ronald Leuchter, Ilana Cass

Objectives: Women with BRCA mutations have an elevated risk of developing breast cancer and epithelial ovarian cancer (EOC) (ovary, fallopian tube, and peritoneal carcinomas). We compared the costs of breast cancer surveillance between BRCA mutation carriers and women without mutations (WT) following a diagnosis of EOC. Methods: IRB approved retrospective review of Women's Cancer Registry identified 360 women with EOC from 1998-2012 who had BRCA genetic testing and subsequent care at our institution. Results: 134 (37%) EOC patients were found to carry a germ-line BRCA 1 or BRCA 2 mutation. 15 women subsequently developed breast cancer: 12 (9%) BRCA patients and 3 (2%) wild-type (WT) patients. BRCA mutation carriers had significantly more annual mammograms (MMG) than WT patients, 78% versus 38%, ($p< 0.0001$). The frequency of breast cancer surveillance in BRCA mutation carriers was unaffected by stage of disease, 87% with early versus 81% with late stage disease. In contrast, WT patients with advanced stage disease had less breast cancer surveillance than early stage patients, 32% versus 76% ($P<0.001$). Other breast cancer surveillance and risk reducing strategies were only used in BRCA mutation carriers: annual breast MRI in 60 patients (45%), >1 breast surgeon consultation in 53 patients (39%), tamoxifen or aromatase inhibitors in 25 (19%) of patients for an average of 12 months. 15 BRCA mutation carriers underwent a prophylactic mastectomy. 75% of breast cancers were detected by either MMG or breast exams and all patients were diagnosed with early

breast cancer (Table 1). MRI did not detect any breast cancers. Surveillance was significantly more costly in BRCA mutation carriers than in WT patients, $512 vs. $261 to detect 1 cancer/per year (p = 0.0007).Conclusions: Breast cancer is more common in BRCA mutation carriers than WT patients following EOC although the incidence is low. MMG detected most breast cancers at an early stage. Multimodality breast cancer surveillance used in BRCA mutation carriers was twice the cost of annual MMG in WT patients. Multimodality breast cancer surveillance should be reconsidered in BRCA mutation carriers after EOC given the significant additional cost and low rates of detection.

Risk of Breast Cancer Following Ovarian Cancer and the Impact on Overall Survival

Daniel Paik, Farin Amersi, Catherine Bresee, Alexandra Gangi, Beth Karlan, Andrew Li, Christine Walsh, BJ Rimel, Ronald Leuchter, Ilana Cass

Objectives: BRCA mutation carriers are at increased risk of breast cancer and epithelial ovarian cancer (EOC) including ovary, fallopian tube and peritoneal cancers. Cancer surveillance guidelines for these high-risk women exist although the risk and rationale for breast cancer surveillance after EOC in BRCA mutation carriers is not well established. We sought to determine the risk and outcome of breast cancer after a diagnosis of EOC. Methods: IRB approved retrospective review of Women's Cancer Registries identified 360 women with EOC from 1998 - 2012 who had BRCA genetic testing and subsequent care at our institution. Results: 134 patients (37%) had a BRCA mutation: 100 BRCA1, 33 BRCA2, 1 BRCA1 and 2 mutation. BRCA mutation carriers were younger than wild type patients (WT) at EOC diagnosis (52 yrs vs. 62 yrs, p<0.001). Most patients had advanced stage disease: 90% BRCA, 80% WT. BRCA mutation carriers were more likely to receive chemotherapy than WT patient, 99% vs. 93% (p=0.004). They were more likely to develop breast cancer than WT patients, after adjusting for age [HR 10.6, p=0.0026]. 15 patients (12 BRCA, 3 WT) developed breast cancer at a median of 3 years after EOC diagnosis. All breast cancers were early stage: 4 DCIS, 6 stage I, 5 stage II. Six women received chemotherapy for their breast cancer. Patients with BRCA-associated EOC had a lower risk of death than WT EOC with a median survival of 10 years vs. 5 years, [HR 0.6, p=0.001]. EOC specific survival between BRCA mutation carriers and WT was 73% and 50% at 5-years and 50% and 27% at 10-years, respectively. There were 185 deaths and 96% were due to EOC in both BRCA mutation carriers and WT patients. There was one death in a WT EOC patient due to breast cancer. There was no difference in overall survival rates between cases with breast cancer and without after co-varying for age and stage of EOC at time of diagnosis and BRCA status (p=0.1).Conclusions: Women with BRCA-associated EOC have a better outcome and a higher risk of subsequent breast cancer than WT EOC patients. Overall survival was dominated by EOC-related mortality. Breast cancer surveillance recommendations in women with BRCA-associated EOC should be balanced by the minimal impact of breast cancer on survival. There is limited utility for breast cancer surveillance in WT patients following a diagnosis of late stage EOC.

A novel clinical trial recruitment strategy for women's cancers

BJ Rimel, Jenny Lester, Catherine Dang, Leah Sabacan, Diane Park, Candice Daneshvar, Beth Karlan

Objective: National clinical trial enrollment in cancer studies remains less than 4%. To address the impediments to trial participation, we developed a novel online registry of women interested in clinical research as well as a clinical trial matching mechanism. We sought to improve clinical trial accrual at a single institution using this strategy to match potentially eligible women with open studies. Methods: Utilizing a secure online verification platform (Docusign) for informed consent, we designed a web based registry for women over age 18 who expressed interest in clinical research. Women could enroll and provide brief clinical and demographic information remotely to aid in determining if they were eligible for an open study at our institution. The registry was IRB approved and the registry website was linked to other portals including the hospital's and, several women's cancers support organizations' websites, and also posted to several social media outlets. Results: In the first 5.5 months, 225 participants enrolled online for an average of 41 participants per month, almost 8 times the rate of accrual compared to an older paper-based system. In addition, since the implementation, 184 participants have already been identified as qualifying for at least one research study available at our institution and 41 of these qualified for more than one study. A total of seven (4%) have already consented thus far and study staff are in the process of working with 20 other participants (total of 14.6%) to set up face-to-face consenting for appropriate studies. Conclusion: Clinical trial enrollment was significantly improved by implementation of an online registry to aid matching of relevant studies to interested patients. In the first six months since launching the web-based portal, 4 fold improvement was seen over our previous enrollment of less than 1%.

Cancer Biology and Therapeutics

A mouse model of epithelial ovarian cancer with defined oncogenic drivers

Hasmik Agadjanian, Dong-Joo Cheon, Elena Diaz, Anna Laury, Carl Miller, Beth Karlan, Sandra Orsulic, Christine Walsh

Epithelial ovarian cancers (EOC) represent the gynecologic malignancy with the highest mortality rate. The Cancer Genome Atlas demonstrated a high degree of genetic heterogeneity among the papillary serous (PS) tumors, the most lethal of the EOC subtypes. Aside from almost universal mutation in TP53, there was a low prevalence of recurrent mutations in other genes, presenting a challenge to the development of targeted therapeutics against this aggressive tumor type. We are developing a mouse model of EOC by introducing defined genetic alterations to mouse ovarian surface epithelial (MOSE) cells. We had a particular interest in developing a cyclin E amplified tumor model, as CCNE1 amplification is found in 20% of human EOC tumors, making this one of the most prevalent PS EOC subtypes. We infected MOSE cells from p53-/- mice with different combinations of oncogenes encoded in retroviral vectors including CCNE1, myc and H-RAS. MOSE cells were infected with one or two oncogenes, passaged and injected into the peritoneal cavity of nude mice. Myc-HRAS caused the rapid development of hemorrhagic ascites and intraperitoneal carcinomatosis in 5 of 5 mice by day 18. HRAS alone and HRAS-CCNE1 both caused intraperitoneal and omental tumors to develop with clear ascites by day 38 in 5 of 5 mice. Myc caused bloating due to the development of frank hemoperitoneum in all 5 mice between days 101 and 150. Only 4 of the 5 mice had small volume intraperitoneal tumors. Myc-CCNE1 similarly caused bloating due to hemorrhagic ascites in 5 of 5 mice by day 70. Histology of the Myc-HRAS, HRAS, HRAS-CCNE1, Myc and Myc-CCNE1 tumors were all consistent with high-grade undifferentiated carcinoma. Tumor cells generated in the Myc-HRAS, HRAS, HRAS-CCNE1, and Myc experiments were capable of causing intraperitoneal tumor development in immunocompetent C57BL/6 mice by day 17, 29 , 54, and 115 respectively. Taken together, our data demonstrate a reproducible mouse model for the development of high-grade epithelial ovarian carcinomas with defined genetic alterations that are capable of study in both immunodeficient and immunocompetent mice. Tumor phenotypes varied based on the combination of activated oncogenes.

T7-pol enhances the oncolethality of targeted siRNA delivery

Felix Alonso-Valenteen, Jay Lubow, Jessica Sims, Michael Taguiam, Chris Hanson, Mitra Mastali, Lali Medina-Kawe

We have previously developed the recombinant fusion protein, HerPBK10, for ligand-directed targeting and penetration of HER2+ breast cancer. HerPBK10 contains a ten lysine (K10) carboxy [C] -terminal tail that allows it to non-covalently bind anionic payloads, such as nucleic acids, by electrophilic interaction. In this study, a modified siRNA capable of both gene silencing and immunostimulation is the payload.

Here, we test the hypothesis that ligand-directed, protein-based nanoparticles delivering siRNA launch a three-pronged attack on tumors by combining missile-like targeting (known as “transductional targeting”) with siRNA-mediated gene silencing, and cytokine-mediated cell death. The latter effect will be accomplished by taking advantage of the normally undesirable cytokine-mediated cytotoxicity induced by epigenetic modifications on certain forms of siRNA, and delivering this to tumors. Here we will deliver siRNA produced by T7 phage polymerase transcription, which introduces a 5’ triphosphate cap that triggers cytokine-mediated toxicity within host cells. So far, in vitro data has shown that this approach is more effective in reducing cell proliferation than synthetic siRNA against the same gene. Furthermore, in vivo xenograft data shows that this strategy can control and ablate the growth of HER2+ tumors. Future directions include IHC investigation of resected tumors and testing this hypothesis in an immunocompetent in vivo setting where we envision this immune-sensitive strategy having even greater efficacy.

YAP1 Is a Key Component of the AR Signaling Complexes in Prostate Cancer Cells

Ahmet Alptekin, Bekir Cinar, Gamze Kuser-Abali

Androgen receptor (AR) plays a central role in prostate tumor promotion and metastasis. Here, we investigated the impact of the YAP1 oncoprotein, a prominent nuclear effector of the Hippo tumor suppressor pathway, on AR transactivation functions and prostate cancer cell growth. YAP1 knockdown attenuated prostate cancer cell growth. Co-immunoprecipitation (Co-IP) and western blot experiments revealed that YAP1 protein is associated with AR protein complexes in cell nuclei, which stimulated by androgen. GST- pulldown experiments further demonstrated recombinant YAP1 protein physically interacted with AR protein complexes. The promoter reporter analysis showed that enforced YAP1 expression promoted the activation of AR-responsive genes and the carboxyl-terminal site of YAP1, which consists of transactivation, PDZ, and coiled-coiled protein-protein interaction domains, appeared to be responsible for the AR transactivation. As revealed by RT-qPCR, disruption of YAP signaling by Mst1 (hippo) induction and RNAi, attenuated the expression of AR-responsive genes selectively. Chromatin-IP and qPCR experiments demonstrated that YAP1 interacted with the Androgen Reponsive Elements (ARE) in PSA promoter, and androgen stimulated this binding. These findings indicate that YAP1 is a binding partner and physiologic regulator of AR and suggest that YAP1 signaling plays a critical role in prostate tumor cell growth and can be targeted for therapeutic interventions.

The role of sphingosine kinase-1 in ovarian cancer progression and the tumor microenvironment

Paul-Joseph Aspuria, Dong-Joo Cheon, Maricel Gozo, Beth Karlan, Sandra Orsulic, Jessica Beach

Sphingosine kinase-1 (SPHK1) is an enzyme that catalyzes the formation of the prosurvival second messenger sphingosine-1-phosphate (S1P) from the proapoptotic lipid sphingosine. Here we report that SPHK1 is overexpressed in a subset of epithelial ovarian cancers (EOC) and correlates with poor

progression-free survival. Overexpression and knockdown of SPHK1 in multiple human EOC cell lines modulates in vitro cell proliferation, anchorage-independent growth, and chemosensitivity. In mouse xenograft studies, intraperitoneal administration of a SPHK1-specific inhibitor (SKI-5c) decreases tumor size, indicating that SPHK1 may be a potential therapeutic target in EOC. S1P has been shown to influence the tumor microenvironment of other cancers; however its role in EOC has not been fully established. Using a co-culture model, we show that overexpression of SPHK1 in EOC cells stimulates the transition of ovarian stromal fibroblasts to myofibroblasts and enhances stromal SPHK1 expression. Biostatistical analysis suggests a significant correlation between SPHK1 expression in EOC cells and the expression of several extracellular matrix genes (FN1, POSTN, VCAN), which have been shown to have role in the EOC tumor microenvironment. Overall, these results suggest that SPHK1 is a critical regulator of ovarian tumor cell proliferation and survival, and a mediator of tumor-stroma interaction.

Succinate dehydrogenase inhibition leads to epithelial-mesenchymal transition and reprogrammed carbon metabolism in mouse ovarian cancer cells

Jessica Beach, Paul-Joseph Aspuria, Sophia Lunt, Leif Varemo, Laurent Vergnes, Maricel Gozo, Brenda Salumbides, Karen Reue, Jens Nielsen, Beth Karlan, Sandra Orsulic

Succinate dehydrogenase (SDH) is a mitochondrial tumor suppressor found altered in various hereditary cancers.We identified that dysregulation of several members involved in SDH function also occurs in serous ovarian cancer, particularly the SDH subunit SDHB. Targeted knockdown of SdhB by shRNA in genetically-defined mouse ovarian cancer cells resulted in enhanced proliferation, increased anchorage independent growth, and epithelial-mesenchymal transition (EMT). Bioinformatic analysis revealed that loss of SdhB led to a transcriptional upregulation of genes involved in metabolic networks affecting histone methylation. We confirmed that loss of SdhB resulted in a hypermethylated epigenome and that increased methylation of H3K27 was sufficient to induce EMT. In addition to these epigenetic effects, loss of SdhB resulted in reprogrammed carbon source utilization and mitochondrial dysfunction. The altered metabolic state of SdhB knockdown cells could be exploited by carbon source starvation or with the anti-diabetic drug metformin. These data point to a novel mechanism wherein modulation of a single metabolic gene influences the epigenetic and metabolic landscape leading to targetable vulnerabilities in ovarian cancer.

Glioma-associated somatic mutations drive a rapid conversion of neural stem cells into tumorigenic oligodendroglial progenitors

Joshua Breunig, Rachel Levy, Jessica Molina, Gi Bum Kim, Moise Danielpour

Introduction: Recent findings regarding the cell (or cells) of origin in brain tumors have yielded conflicting results. Specifically, several studies have indicated that glioma stem cells show remarkable similarity to neural stem cells (NSCs). Alternatively, a large body of evidence by multiple groups has

shown that in many cases the tumor cell of origin is a rapidly proliferating oligodendrocyte progenitor cell (OPC)-like population. Method: We have created a novel, autochthonic, in vivo model of high grade oligodendroglioma which allows for exquisite control over the genetic determinants as well as the temporal and spatial genesis of glioma, permitting for greater insight into the cellular dynamics of tumor initiation. Somatic mutations of the RTK/Ras pathway are introduced by neonatal electroporation of plasmid DNA in combination with transposon technology. Results: Within 48 hours, transgenes are expressed at a high level with coincident fluorescent protein labeling. Notably, starting at these early time points and proceeding through the first week, radial glial stem cells transform into antigenically-defined oligodendroglial-like progenitor cells, prematurely depleting the neural stem cell population. Conclusions: Thus, we conclude that naturally-occurring somatic mutations drive a rapid and virtually complete conversion of NSCs into an OPC phenotype in a manner that would be difficult to observe in other models, thus unifying the previously disparate findings.

The role of Olig2 in glioma initiation and progression

Joshua Breunig, Rachel Levy, Jessica Molina, Gi Bum Kim, Moise Danielpour, Hannah Park

Introduction: Recent findings have suggested that Olig genes are essential for the proliferation of tumor cells and progression of glioma. Specifically, it has been documented that Olig genes are important for tumor cell proliferation and resistance to p53 responses to genotoxicity. Method: We have created a new model of high grade glioma, which displays ubiquitous expression of Olig2 and other oligodendrocyte markers. This model allows for co-expression of any plasmid. Employing this advantage, we employed a dominant negative form of Olig2 to assess the contribution of Olig2 repressor function to glioma initiation and propagation. Further, we have created shRNAs targeting Olig2. The ability of these constructs to attenuate Olig2 expression and glioma growth will be explored. Results: Notably, expression of this DN-Olig2 failed to prevent tumor proliferation and invasion. Instead, the tumor cells converted from oligodendroglia-type progenitors into immature astroglial lineage tumor progenitors. shRNA validation is currently underway. Conclusions: Dominant negative findings suggest that targeting Olig genes to treat tumors may be ineffective as gliomas can utilize Olig2 independent pathways for propagation by changing lineages. However, more investigation--including Olig2 shRNA treatment of glioma--needs to be done regarding the possible function of Olig2 as a transcriptional activator in this context.

Targeting fatty acid synthesis in gliomagenesis

Joshua Breunig, Rachel Levy, Jessica Molina, Gi Bum Kim, Moise Danielpour, Hannah Park

Pediatric brain tumors are the most common solid tumor of childhood and the leading cause of solid tumor cancer deaths in those under 20. Therapeutic development is critically important, because current standard-of-care treatment of pediatric brain tumors (e.g., surgery, radiation and

chemotherapy) carries great risk of brain injury with long-term neurological, intellectual, and psychological damage. We have created a new rodent model of pediatric brain tumors based on in vivo expression of human mutations, using a technique called electroporation. Further, this model allows for facile isolation and genetic analysis of tumor cells. Using genome-wide expression analysis we have noted evidence for their increased reliance on a specific metabolic pathway-fatty acid synthesis. Interestingly, most normal cells in the body do not naturally synthesize fatty acids, relying on circulating lipids, while tumor cells are thought to require fatty acid synthesis for growth and proliferation. We have found upregulation of Fasn protein in glioma progenitors in vivo. Further, pharmacological inhibition of Fasn decreased the growth of glioma progenitors. Given the necessity for new, targeted therapeutics to combat this devastating disease, we aim to exploit our model as a pre-clinical testing platform to investigate the efficacy of fatty acid synthesis blockade as a treatment for brain cancer. We have now created Fasn-targeting shRNA's and are investigating their efficacy in impeding tumor progression in vivo.

Non-serous ovarian carcinomas express low levels of ASS1: Implications for arginine depletion therapy

Dong-Joo Cheon, Christine Walsh, John Bomalaski, Ann Walts, Beth Karlan, Sandra Orsulic

Arginine-depletion therapy with pegylated arginine deiminase (ADI-PEG 20) has been effective in several cancer types that exhibit low levels of arginine succinate synthetase(ASS1), including hepatocellular carcinoma, melanoma, and prostate cancer. Ovarian cancer has been considered unsuitable for arginine-depletion therapy due to a high expression of ASS1. However, all ASS1 expression data in ovarian cancer have been obtained from the serous subtype, not from the other major histotypes. The goal of this study was to assess the levels of ASS1 in four major histotypes (serous, endometrioid, mucinous, clear cell) of ovarian cancer to define patient groups that could benefit from arginine depletion therapy.ASS1 mRNA and protein levels were examined in a large cohort of primary ovarian cancers and ovarian cancer cell lines using public datasets and immunohistochemistry. We determined that while the majority of serous ovarian cancers express high levels of ASS1, non-serous ovarian cancers exhibit low levels of ASS1. The in vitro sensitivity of ovarian cancer cell lines to arginine depletion with ADI-PEG20 was inversely correlated with ASS1 expression.Our data suggest that a subset of non-serous ovarian cancer subtypes is auxotrophic for arginine and should be considered for clinical trials with ADI-PEG 20.

KRAS hotspot mutation correlates with MLH1 methylation in endometrial carcinomas with microsatellite instability: a potential triage tool for Lynch Syndrome evaluation

Joshua Cohen, Wolf Wiedemeyer, Beth Karlan, Christine Walsh

Objective: In colorectal carcinomas with microsatellite instability (MSI), the presence of a BRAF V600E mutation is highly predictive of sporadic MLH1 methylation and the absence Lynch Syndrome. Our

objective was to determine whether a similar predictor exists in endometrial carcinoma. Methods: Data from endometrial carcinoma patients profiled by The Cancer Genome Atlas (TCGA) was downloaded from CBioPortal. Somatic gene mutation rates were analyzed and compared among MSI-high (MSI-H) tumors with and without MLH1 methylation. Standard statistical tests were applied. Results: Among 245 endometrial carcinomas, 73 (29%) were MSI-H. All MSI-H tumors were endometrioid (100%), grade was evenly distributed (28% G1, 33% G2, 38% G3) and the majority were early stage (79% stage I). 67 of 73 (92%) MSI-H tumors had MLH1 methylation. Among 11 genes with a somatic mutation rate of >20% in MSI-H tumors, only ARID1A, KRAS, PIK3R1 and PTEN were mutated at a higher rate in tumors with MLH1 methylation and only KRAS demonstrated a hotspot for recurrent mutations. Among 23 tumors with a KRAS codon 12 or 13 (C12/13) mutation, all 23 (100%) had MLH1 methylation. In this group, somatic mutations in a Lynch Syndrome (LS) gene (MLH1, MSH2, MSH6 or PMS2) were found in 13% (3 of 23). Among 6 tumors with no KRAS C12/13 mutation and no MLH1 methylation, somatic mutations in a LS gene were found in 83% (5 of 6); see figure. Mutations in BRAF were infrequent in endometrial carcinoma. Conclusions: A KRAS hotspot mutation in codon 12 or 13 correlates highly with MLH1 methylation in the endometrial cancer dataset from TCGA. Further work is necessary to determine the germline mutation status of the Lynch Syndrome genes in these cases. Absence of germline LS mutations would suggest KRAS testing as a potential triage tool to identify sporadic cases in the work-up of endometrial carcinomas with microsatellite instability. This could avoid the expense of full gene sequencing of the Lynch Syndrome genes.

Digital Image Analysis meets 'OMICS'

Andrew Conley, Arkadiusz Gertych, Sanica Bhele, Sambit Mohanty, Hyung Kim, Mahul Amin, Beatrice Knudsen

Clear cell renal cell carcinoma (CCRCC) is graded using nuclear morphology into Fuhrman grades 1-4, which are predictive of patient prognosis. Despite the recent accumulation of CCRCC data through The Cancer Genome Atlas (TCGA), the genomic and epigenetic changes that determine the morphometric features of the Fuhrman grade remain poorly understood. Here we use computational image analysis techniques for the high-though put identification of nuclei. Further, we use supervised machine learning and the extracted features to distinguish between nuclei from cancer and normal cells in the tumor section. Our unique image analysis approach will provide new opportunities to quantify percentages of several normal cell types in the tumor microenvironment. By adjusting for normal cell types, we will significantly improve the detection of copy number alteration, DNA methylation, and mRNA expression specific to the cancer cells in TCGA data. Thus, integration of image analysis, nuclear classification, and genomic data will allow for deconvolution of the contribution of different cell types and more precisely identify the underlying genomic, transcriptomic and epigenetic changes that are associated with the Fuhrman grade and predictive of the risk of tumor recurrence.

IDH1 modulates the immune response to glioblastoma

Ryan Cordner, Michelle Jhun, Akanksha Panwar, HongQiang Wang, Nicole Yeager, Joseph McAbee, Armen Mardiros, Akane Takei, Xuemo Fan, Keith Black, Christopher Wheeler

Dendritic cell (DC) vaccines for glioblastoma (GBM) can effectively extend overall patient survival, but they are not curative. Isocitrate Dehydrogenase 1 (IDH1) mutations are associated with better overall survival in glioblastoma patients, but their effects on DC vaccine outcomes are unknown. Alloreactive CD8+ hybridoma cells exhibited delayed lysis of GL26 glioma cells expressing mutant IDH1, and significantly increased lysis of GL26 transfected with wild-type IDH1. We therefore examined the possibility that IDH1 possessed a novel, immune-specific function. Molecular modeling analysis revealed that sialic acid stably binds the IDH1 catalytic site where isocitrate is normally found, whereas the common IDH1 mutation, R132H, was predicted to bindsialic acid with 100-fold lower affinity. Since IDH exhibits catalytic site structural similarity to microbial neuraminidase, which de-sialylates T cells to enhance their antigen reactivity, we further examined IDH1 possessed novel sialidase function. Invitro tests confirmed that IDH1 has sialidase activity against glycoprotein only in the presence of NADP+. Physiologic quantities of IDH1 were also found to associate specifically with a surface heterodimer identified as the CD8 coreceptor in mouse T cell lysates. Flow cytometric analysis of IDH1 treated splenocytes revealed increased PNA binding on CD8+ cells. Finally, IDH1 treatment greatly increased the amount of IFN-γ produced in response to various pMHC I tumor epitopes, and R132H IDH1 transfection of GL26 abrogated the longer survival conferred by DC vaccination relative to control transfected GL26. Together, these results suggest thatwild-type IDH1possesses novel CD8 T cell-specific binding and sialidase functions that enhance CTL epitope binding and tumor target lysis. In contrast, R132H IDH1 appears to delay immune lysis of glioma cells expressing it, and abrogates survival benefit conferred by DC vaccination. Thus, the novel sialidase function of IDH1 may be a useful target to improve the clinical benefits of DC vaccine therapy.

Differential Radiosensitivity and Clonal Behavior of Airway Epithelial Progenitors

Alicia Farin, Craig Rackley, Nicholas Manzo, Barry Stripp

Environmental or therapeutic exposure of lung tissue to ionizing radiation contributes to tissue remodeling and an increase in cancer risk. In other organ systems such as the gut, exposure to IR has effects that differ between cell types, with radiation-resistant progenitors contributing to tissue regeneration after sub-lethal exposure. However, little is known of radiation effects on lung cells that may impact tissue homeostasis and remodeling. We hypothesize that regional airway epithelial progenitor cells will show differences in their susceptibility to radiation injury, and that radiation exposure will lead to changes in the clonal behavior of surviving progenitors. To observe the cell-intrinsic effects of ionizing radiation in the lung, we isolated and irradiated normal human proximal basal cells and plated them in a 3D sphere-forming assay. We observed a decrease in colony forming efficiency (CFE) with increasing dose of ionizing radiation. A combination of in vivo and in vitro assays were used to evaluate radio-sensitivity of epithelial progenitor cells in mouse airways. Whole-body radiation exposure

resulted in the appearance of patches of clonally-derived progeny suggestive of the presence of highly clonogenic radio-resistant cells. To understand the effects of radiation on different subsets of epithelial progenitors in mouse airways we isolated Sca1+ and Sca1- cell fractions and evaluated their behavior in a 3D sphere-forming assay. We observed an accelerated rate of decrease in CFE in the Sca1- fraction as compared to the Sca1+ fraction, demonstrating that distinct progenitor cell populations differ in their radio-sensitivity. A microarray analysis was performed to compare gene expression between regionally distinct populations of cells in the normal human lung. We found that genes from the pro-apoptotic death associated protein kinase (DAPK) family were significantly differentially expressed between proximal and distal epithelial progenitor cells. DAPK1 and DAPK2 had higher expression in distal basal and alveolar type 2 cells as compared to proximal basal cells. Future studies are aimed at defining mechanisms of radio-resistance within airway progenitor cells with a focus on roles for DAPK.

Tissue microarray construction and image analysis to develop predictive biomarkers for targeted therapies across multiple cancer types

Arkadiusz Gertych, Zhaoxuan Ma, Youan Xiaopu, Luz Felix, Steven Swartwood, Beatrice Knudsen

Background: MET/HGF pathway is involved in cancer growth and progression and is a target for drug development. However, it is unclear which cancer types respond to MET inhibitors because the MET activation, which includes HGF stimulation, overexpression, mutation and receptor crosstalk, is difficult to determine. Recent data demonstrate that the activity of Src-family kinases (SFK) causes resistance to MET inhibitory drugs. Therefore, identifying cancers that potentially respond to anti-MET drugs will require the development of a multi-biomarker panel consisting of protein expression and phosphorylation measurements. Methods: We constructed tissue microarray (TMA) slides, which include 15 cancers and 20 cases per cancer type. TMAs were stained with 3 MET antibodies used in clinical trials, and separately with pMET, HGF and pSrc. Quantitative expression was obtained by digital image analysis. Results: The 3 MET antibodies specifically detect the MET protein. However, differences in sensitivity led to different staining patterns. MET expression was highest in cancer, whereas HGF was primarily expressed in stroma. The data will be used to determine whether the MET/HGF expressions correlates with pMET phosphorylation status, and to study the co-occurrence of pSFK with pMET.Conclusion: Results from this first large scale IHC study of MET activation will suggest which cancers should be responsive to MET kinases inhibitors and which cancers could develop rapid drug resistance due to expression of pSFK. The data will reveal whether MET inhibitors can be used for all MET expressing cancers, or only in certain cancer types.

FOXC1 regulates Cancer Stem Cells Properties via inducing SMO-independent Gli activation and confers anti-Hedgehog drug resistance in Basal-like Breast Cancer

Bingchen Han, Yangli Jin, Shikha Bose, Xiao Zhang, Ying Qu, Beth Karlan, Armando Giuliano, XiaoJiang Cui

Basal-like breast cancer (BLBC), an aggressive subtype of breast cancer, are usually associated with high histologic grade, high recurrence rate, short recurrence-free survival, younger patient age, poor outcome, and a propensity to metastasize to the brain and lung. So far, developing targeted therapies against BLBC still remains a great challenge. Uncovering the underlying molecular events and identify therapeutic target becomes highly urgent. Forkhead Box C1 (FOXC1), a biomarker for BLBC, is associated with poor prognosis in patients with BLBC. Here, we found that FOXC1 increases cancer stem cells (CSCs) properties in BLBC cells in vitro and in vivo. FOXC1 activates SMO-independent non-canonical Hedgehog (Hh) signaling pathway, which mediates the effects of FOXC1 on CSCs properties in BLBC cells. Further study showed that the N-terminal of FOXC1 bind directly to the Gli2 transcription factor, which has the highest transcriptional activity in Hh signaling pathway. Here we also found that the elevated expression of FOXC1 induces anti-Hh pathway drug resistance. All of these data demonstrate a novel mechanism underlying the regulation of CSC properties and the poor prognosis of BLBC patients, and may provide new insight into anti-CSC therapy resistance. Thus, our study provides a strong rationale for developing FOXC1-targeted therapy for treating BLBC patients.

Enhancing Dendritic Cell Vaccines by Stimulating TLR9 and Dectin-1

Nargess Hassanzadeh-Kiabi, Yanping Wang, Hyung Kim, Helen Goodridge

Dendritic cells (DCs) are key players in linking the innate and adaptive immune systems. Unfortunately, the ability of DCs to activate T cells and propagate an anti-tumor response is blocked by the suppressive microenvironment created by the tumor. To overcome this suppression DC vaccine therapies are an attractive area of study. Previous studies have shown that TLR and Dectin-1 agonists activate DCs and have collaborative effects. Both CpG (TLR9 ligand) and β-glucan (Dectin-1 ligand) have been shown separately to have anti-tumor effects in vivo. Therefore, we propose to use CpG and β-glucan together to activate DCs in vitro to produce an improved DC vaccine that induces a more robust in vivo anti-tumor response. Normally, DCs are matured with GM-CSF, which increases their antigen uptake capacity and cross-presentation ability. However, it has also been shown that bone marrow DCs grown in the presence of GM-CSF are able to expand Foxp3+ Tregs. Interestingly, when we stimulated DCs with CpG/β-glucan in the absence of GM-CSF, their cytokine production was reduced, but they were better able to stimulate CD4+ T cells to proliferate compared with DCs stimulated in the presence of GM-CSF. Finally, we show that DCs co-stimulated with CpG and β-glucan in the absence of GM-CSF were able to prevent tumor growth in vivo.

Which cancers respond to drugs that inhibit the MET receptor?

Fangjin Huang, Xiaopu Yuan, Sanica Bhele, Mariza Venturina, Arkadiusz Gertych, Beatrice Knudsen

Background: More than 20 companies are developing drugs against MET, a receptor tyrosine kinase on the cell surface that binds the ligand, Hepatocyte Growth Factor (HGF). The greatest drug response occurs in cancers with active MET (pMET). Here, we use large public datasets to identify patterns of MET receptor activation across cancer types. Methods: We analyzed cell lines with known MET phosphorylation status, the Cancer Cell Line Encyclopedia (CCLE) and Sanger Institute Cell line compendium and TCGA datasets from 11 cancer types. We used Spearman's rank correlation coefficient to calculate the associations and used rank product method to calculate the concordance between the expressions of the proteins and mRNA. Outliers were determined by the method from Box-and-Whisker Plots. Results: We identified all 3 mechanisms of MET activation in carcinomas: (1) Autocrine HGF secretion was detected in 4.3% of carcinoma cell lines in CCLE. High HGF RNA was present in 0.71% of cancers in TCGA and 1/3 of these cancers also expressed pMET. (2) MET overexpression, was evaluated through RNA and protein expression in renal and colon cancer. MET RNA and protein are correlated in renal cancer ($\sigma = 0.48$), but not in colon cancer ($\sigma = -0.05$), suggesting differences the mechanism of MET overexpression in different cancer types. (3) Most frequently, MET activation occurred through receptor crosstalk based on coexpression of pHER3-Y1298 and pEGFR-Y1173 with pMET-Y1235. Conclusion: While frequencies of MET activation are fairly consistent across cancer types, the mechanisms through which MET becomes activated, differ. This insight demonstrates the need for cancer type specific treatment strategies and the development of cancer-type specific biomarker panels to measure the activation state of MET.

Transcriptional Silencing of Mst1/Hippo by a Coordinated MYC and EZH2 Signaling in Prostate Cancer

Gamze Kuser Abali, Ahmet Alptekin, Bekir Cinar

Prostate cancer is a major cause of cancer-related death in western countries because of the poorly understood disease mechanisms. Hippo-like MST1 protein kinase regulates cell growth, stem cell self-renewal and carcinogenesis. The loss of Mst1 expression is implicated in the etiology several types of cancers with poor prognosis, including prostate cancer. However, the underlying mechanism of Mst1 transcriptional downregulation in prostate cancer is unknown. Here, we report that MYC and EZH2 function as epigenetic suppressors of MST1 in human prostate cancer cells. We showed that decline in MST1 expression correlated with EZH2 and MYC upregulation as demonstrated by reverse-transcriptase quantitative polymerase chain reaction (RT-qPCR) and western blot. Using methylation sensitive PCR and bisulfite genomic DNA sequencing, we showed that Mst1 promoter DNA was methylated at CpG sites that led to a reduction in MST1 expression. Our pharmacologic or RNAi experiments revealed that EZH2 contributed to MST1 silencing by inducing MST1 promoter DNA methylation and H3K27me3 modification that was accompanied by a reduced H3K4me3 mark and RNA polymerase II occupancy on MST1 promoter and thereby reducing its promoter activity and expression. On the other hand, overexpression of MYC stimulated EZH2 and conversely reduced MST1 promoter activation and its

mRNA levels. Potent EZH2 inhibitors reversed the MYC effects on MST1, indicating that MYC attenuates MST1 through EZH2. Moreover, MST1 knockdown caused cell resistance to androgen deficiency and to EZH2, or MYC inhibitor-induced growth retardation. These findings indicate that MYC cooperates with EZH2 to epigenetically silence MST1 and suggest that the loss of MST1/Hippo is critical for MYC- and EZH2-driven cancer cell survival.

Whole-Transcriptome profiling of formalin-fixed, paraffin-embedded clear cell renal cell carcinoma by RNAseq

Ping Li, Hao Zhang, Lindsay Spurka, Jordan Brown, Jie Tang, Quoclinh Nguyen, Vincent Funari, Hyung Kim

Background: Deep sequencing of RNA is rapidly becoming the standard for profiling the entire transcriptome. However formalin-fixed paraffin-embedded (FFPE) ccRCC samples have not been widely used due to the low quality and quantity of RNA from archival tissue. Methods/Results: We optimized strategies for removing ribosomal RNA and RNAseq library preparation using FFPE tissue for whole transcriptome profiling; we carried out whole-transcriptome mRNAseq analysis using two matching pairs of fresh frozen (FF) and FFPE ccRCC tumor tissue. Studies performed on paired frozen and FFPE specimens showed very similar results (r=0.919 for tumor 1 and r=0.954 for tumor 2). We also performed qPCR on the Taqman OpenArray® NT Cycler System (OA), which provides for high-throughput, nanoliter-scale qPCR reactions. The q-PCR data showed a high correlation with the data from RNA-Seq. (r=0.813 for tumor 1 FF; r=0.775 for tumor 1 FFPE; r=0.815 for tumor 2 FF; r=0.803 for tumor 2 FFPE) We did secondary analysis of The Cancer Genome Atlas (TCGA) data after incorporating our RNA-seq data. Conclusion: This is the first study to perform transcriptome-wide RNA-seq on FFPE RCC. The RNAseq results correlated well to the conventional qPCR data and can be used for discovery and validation of biomarker.

Targeting SREBP by Fatostatin in Metastatic Castration-Resistant Prostate Cancer Cells: Effects on Cell Proliferation, Cell Cycle and Tumor Growth

Xiangyan Li, Yi-Ting Chen, Wen-Chin Huang

Resistance to current therapeutic agents is a major challenge in treatment of lethal metastatic castration-resistant prostate cancer (mCRPC). SREBPs, sterol regulatory element-binding proteins, and their regulated downstream metabolic and signaling networks play important roles in prostate cancer (PCa) development and CRPC progression. Here, we demonstrated that impaired SREBP activity by fatostatin, a new SREBP inhibitor, significantly suppressed cell proliferation, metastatic capability and causes G2/M cell cycle arrest through the accumulation of p21 in mCRPC cells, PC-3 and DU145. Further, we showed the potent therapeutic efficacy of fatostatin by inhibition of subcutaneous PC-3 tumor growth in a xenografted model with low cytotoxicity. The in vitro and in vivo effects of fatostatin in mCRPC cells were due to blockade of SREBP-regulated metabolic pathways. Together, these promising

data offer the insights that targeting SREBP by fatostatin could be a potential new therapeutic approach for lethal mCRPC.

Effect of Lapatinib in an epidermal growth factor receptor-driven pituitary prolactinoma transgenic mice model

Xiaohai Liu, Maya Kano, Araki Takako

Epidermal growth factor (EGF) regulates pituitary development, hormone synthesis and cell proliferation. We established a EGFR or HER2-driven prolactinoma transgenic mice model, in which pituitaries of the mice were enlarged or pituitary adenomas appeared during the period of 13-15 months. Immunohistochemistry and Western blot confirmed abundant EGFR or Neu protein expression with overexpression of PRL in this model. 100-150 mg/kg oral treatment of Lapatinib, a dual tyrosine kinase inhibitor (TKI) of both epidermal growth factor receptor (EGFR)/ErbB1 and HER2 caused serum PRL suppression and PRL protein decrease in this prolactinoma models. These results demonstrate that EGFR and HER2 potently induces tumorigenesis of prolactinoma. And this receptor could be an effective target for prolactinoma therapy.

Single-CTC whole genome sequencing in prostate cancer

Yi-Tsung Lu, Edwin Posadas

Background: Circulating tumor cells (CTCs) are rare tumor cells shed from a patient's tumors into the circulation. It has been hypothesized that CTCs could be used as a "liquid biopsy" to provide dynamic insight into the biology of an underlying cancer. Compared to conventional tumor biopsies, CTCs can be repeatedly isolated from the peripheral blood in a minimally invasive manner. This would allow physicians to monitor biological variations with high temporal resolution making it possible to characterize the dynamic evolution of a cancer without needing serial biopsies. However, due to the low abundance and technical limitations in enriching CTCs, it had been very difficult to characterize CTCs on a whole genome scale. The surrogacy of CTCs for conventional tumor tissues has never been demonstrated beyond targeted genomic alterations such as shared ETS fusions in prostate cancer (PCa), EGFR mutations in lung cancer and BRAF mutations in melanoma. With recent technical advances in single-cell sequencing and single-CTC isolation technologies, we now have the capability to perform single-CTC whole genome sequencing (WGS). Method: By combining NanoVelcro CTC Chip with laser microdissection, we developed a robust platform for single-CTC isolation. Subsequent WGS was performed after the multiple displacement amplification of our single-CTC DNA. From a prostate cancer patient, we performed WGS on four of his CTCs on two different time point, his primary prostatectomy tissue and liver metastasis along with WBC and normal adjacent tissue from the prostatectomy as controls. Copy number variations, single nucleotide variations (SNVs) and structural variations (SVs), including rearrangements were identified for comparison. Bioinformatic analysis was performed to

demonstrate the surrogacy of CTCs to the tumors as well as the heterogeneity of in this late-stage prostate cancer patient. Results: We successfully performed WGS on isolated single CTCs, achieving 30X depth and >95% coverage. SNV analysis reveals that 33.5% (289/863) of founder somatic mutations (defined as mutations shared between primary and metastatic tumors) can be found in CTCs. Looking into the mutational landscape of CTCs, 48% of the CTC mutations can be traced back to either primary or metastatic tumor. Per SV analysis, no ETS-related fusions are found in this patient. However, we do find and validated a rearrangement in chromosome 3 and an interchromosomal rearrangement between chr13 and chr15. These rearrangements are shared between both tumor tissues and most of the CTCs, but not discovered in WBC and normal adjacent tissue. At the same time, highly heterogeneous short SVs were discovered in PTEN, RB1 and BRCA2 in all tumor samples. Conclusion: We demonstrate the first-in-field capability of performing single-CTC WGS. Based on the high-quality WGS data, we validate the genomic surrogacy of CTCs by the detection of shared SNVs and rearrangements between CTCs and tumor tissues. The heterogeneous mutational landscapes of SNVs and SVs are also discovered in single-CTCs, indicating the value of our single-CTC WGS approach. We envision our single-CTC WGS approach will be utilized for studying tumor heterogeneity and the biological evolution of cancer during disease progression, even during anticancer therapy.

Protein Construct for Tumor Targeting through c-Met

Mitra Mastali, Jessica Sims, Michael Taguiam, Chris Hanson, Mitra Mastali, Lali Medina-Kawe

C-Met is a receptor tyrosine kinase (RTK) that is activated by the binding of its ligand, hepatocyte growth factor (HGF). The HGF/c-Met pathway contributes to embryonic development, wound healing, cell proliferation and survival, motility and morphogenesis. It is also one of the most frequently dysregulated pathways in a variety of human cancers, including breast, ovarian, prostate, lung, and pancreatic. Importantly, cell surface elevation of c-Met has been associated with drug-resistance, including acquired resistance to current signal-blocking therapies. Tumor-targeting strategies that do not require signal inhibition may prove more effective on c-Met positive cancer cells. This may be addressed by ligands that recognize c-Met to trigger cell uptake of attached therapeutics, thus bypassing the need to block signaling. HGF has the potential to accomplish this but its requirement for tetramerization and disulfide bonding presents technical complications for therapeutics development. An alternative, and potentially superior ligand for c-Met targeting may possibly be derived from a bacterium that causes food-poisoning. The human pathogen, Listeria monocytogenes, binds c-Met to invade host cells through its surface proteins called Internalins. Specifically, Internalin B (InlB) triggers receptor-mediated endocytosis after c-Met binding. InlB and HGF recognize different regions of c-Met, and InlB does not require tetramerization or disulfide bonds for binding. Consequently, InlB is more suitable for recombinant protein production than HGF. We have previously shown that PBK10, a recombinant protein derived from the adenovirus capsid penton base, can mediate gene and drug delivery into cells through the membrane penetrating activity of the penton base. Moreover, PBK10 can be targeted to tumor cells via recombinant fusion to tumor-homing ligands. Here, we explore the possibility of fusing the receptor-binding site of InlB to PBK10 for targeting attached cytotoxic agents to c-Met positive

cancer cells, and transporting such agents directly into these cells via receptor-mediated endocytosis and membrane penetration.

Large oncosomes mediate transcription factor alterations in the tumor microenvironment

Valentina Minciacchi, Sungyong You, Matteo Morello, Wei Yang, Mandana Zandian, Mirja Rotinen, Samantha Morley, Michael Freeman, Dolores Di Vizio

Objective: We recently discovered that rapidly migratory, "amoeboid" prostate cancer (PCa) cells shed large (1-10μm diameter), bioactive extracellular vesicles (EV), termed large oncosomes (LO), whose abundance correlates with tumor aggressiveness (Di Vizio et al., Cancer Res. 2009; Di Vizio et al. Am J Pathol. 2012). Increasing evidence indicates that large oncosomes are taken up by recipient cells and can affect signal transduction and alter the behavior of recipient cells in a manner that promotes oncogenesis and tumor progression. In this study, we performed a functional and molecular characterization of tumor-derived LO. Methods: We used high-speed centrifugation and filtration; Immuno-flow cytometry with size beads; confocal microscopy, and quantitative LC-MS/MS SILAC. Results: LO stimulated the migration of tumor and endothelial cells and contained active MMP2 and MMP9, key proteases involved in tumor cell invasion. LC-MS/MS SILAC identified 2074 intracellular and 1542 EV proteins (FDR < 0.05), with 354 EV-specific proteins. A significant enrichment in proteins involved in cell adhesion and migration was found in EV from DU145 cells in which the amoeboid phenotype was accentuated by knocking down the cytoskeleton regulator diaphanous related formin 3 (DIAPH3). A comparative analysis using Gene Ontology (GO) revealed that most of the proteins identified both in LO and smaller EV are involved in cell cycle regulation, vesicle-mediated transport, and cell motility. Internalization of LO into myofibroblasts was observed by confocal microscopy and this process seems to be the result of activation of an endocytic pathway by the recipient cells. Confocal analysis also showed that these structurally intact vesicles localize predominantly in the perinuclear area at early time points, but penetrate into the nucleus at later times. Finally microarray analysis of stromal cells challenged with LO demonstrated altered levels of transcription factors relevant to tumor progression and metastasis as consequence of LO internalization, a possible novel mechanism of EV-mediated intracellular communication. Conclusions: This is the first comprehensive proteomic analysis of LO and also the first time that activation of transcription factors in recipient cells by LO have been observed. These findings suggest the capability of LO to broadly affect the tumor microenvironment. They also highlight the translational potential for tracking of these large, visible EV in assessments of disease status in cancer.

Mechanisms of Resistance to MET-Tyrosine Kinase Inhibitors

Sara Pollan, Nishit Mukhopadhyay, Samantha Morley, Jaime Gard, Lipsa Das, Michael Freeman, Anne Cress, Beatrice Knudsen

Background: This study seeks to identify mechanisms of resistance to inhibitors of the transmembrane tyrosine receptor kinase MET. The MET/HGF receptor/ligand system is overexpressed in metastatic prostate cancer and therefore considered an important drug target. Cabozantinib (XL184), a MET, RON and VEGFR2 inhibitor, demonstrates significant activity in patients with metastatic prostate cancer, however the duration of the treatment effect is only approximately one year. Failure to respond to treatment is cause by cellular resistance to the drug. Known mechanisms of resistance to MET inhibitors include receptor mutations, autonomous activity of downstream pathways such as RAS and PI3K, phosphorylation of the MET c-terminus by SRC family kinases and receptor transactivation. Multi-kinase inhibitors of MET are currently available.Materials and Methods: A kinome siRNA screen was used to identify genes that regulate the response to MET inhibitors, XL184 and SGX523, a specific MET inhibitor. Seven hundred and thirteen siRNAs from a kinome library were screened by the University of Washington siRNA core. S-DU145 (prostate) and H596 (lung) cell lines were selected for analysis. The drug concentration corresponded to the IC30, 30% inhibition of growth. The following groups were evaluated for growth: siRNA - unstimulated, siRNA+ HGF, siRNA+ HGF+ XL154, siRNA+ HGF+ SGX523. Samples were run in triplicate.Results: The kinome screen identified key gene families involved in resistance to XL184 and SGX523. These include receptor-interacting serine/threonine-protein kinases (RIPK1/4), which play a role in necrosis, and microtubule associated serine/threonine kinases (MAST1-4), which presumably regulate the organization of microtubules. An ONCOMINE search confirms the loss of MAST4 in prostate, breast, colon, lung, pancreatic and ovarian samples. Knocking down MAST4 increases SRC and MAPK phosphorylation. To comprehensively interrogate the MAST4 deficient phenotype, we are generating stable MAST4 silenced cells.Future directions: To link MAST4 loss to XL184 resistance, we will use an immunofluorescent assay of tubulin polymerization. The targets that we identified through the siRNA screen show for the first time that drug resistance to tyrosine kinase inhibitors can be caused by cytoskeletal mechanisms. As the actual cause of drug resistance, we postulate endpoints that affect receptor recycling and cell polarization.

Targeting Metabolic Plasticity in Triple-negative Breast Cancer

V Krishnan Ramanujan, Qijin Xu

Mitochondria serve dual roles in cellular metabolism : as the hub of cellular energetics as well as the centers of programmed cell death. Normal cell development and differentiation requires a balanced performance of both these functional aspects of mitochondria. A long-standing question in tumor metabolism is the “metabolic switch” in tumor cells. Proposed by Otto Warburg in 1950s, the metabolic switch pertains to the observation that the tumor cells preferentially depend on the glycolytic pathway and avoid an apparently more efficient mitochondrial pathway even in the presence of oxygen (aerobic glycolysis). There is a general consensus in the field that many tumors indeed display “Warburg

phenotype" and even when the cells isolated from the tumors were cultured in abundant supply of oxygen. This feature is already in clinical use where radioactive glucose analog (2FdG) is used to achieve contrast (owing to increased tumor uptake) in positron emission tomography. Despite the clinical utility and the realization of its ubiquitous nature, a clear mechanistic understanding of the Warburg phenomenon is still lacking. In particular, there is no clarity on what are the molecular players involved in triggering/sustaining metabolic switch in cancer cells although the recent studies on pyruvate kinase M2 (PKM2) seem to offer a good starting point. It is also not known if cancer cells indeed have "dysfunctional" mitochondria (original hypothesis) or if the cancer cells tend to "evade" mitochondrial function owing to tighter regulatory steps in mitochondrial oxidative phosphorylation (OxPhos) than in glycolytic pathway; We believe that two critical steps might contribute to a better understanding of the metabolic switch in cancer aetiology namely: (i) a comprehensive model system for evaluating the effects of systematic perturbations to mitochondrial pathway (mitochondrial reprogramming strategies) on the emergence or reversal of metabolic switch in cancer cells and (ii) a non-invasive method of monitoring the effects of such mitochondrial reprogramming in preclinical animal models of cancer to examine the physiological manifestations and ramifications of the Warburg phenomenon in vivo. Using a model system of triple-negative (human) breast cancer cells and high-resolution imaging strategies, we have started accumulating evidence that the tumorigenic/metabolic characteristics can be strategically modulated via targeting mitochondrial pathways in triple negative breast cancer cells and we will present these preliminary findings in the conference poster.

Oncogenic regulation of DIAPH3, a frequently deleted gene in prostate cancer and a repressor of the mesenchymal-amoeboid transition

Mirja Rotinen, Sungyong You, Samantha Morley, Julie Yang, Kenneth Steadman, Dolores DiVizio, Jayoung Kim, Beatrice Knudsen, Ignacio Encio, Michael Freeman

Introduction and objectives: Formin family proteins regulate cytoskeletal organization, a process that affects tumor progression. Within this family, Diaphanous-related formin-3 (DIAPH3) has been described by us as a non-canonical regulator of metastasis that restrains conversion to amoeboid cell behavior in multiple cancer types (Hager et al, EMBO Mol Med 2012; Di Vizio et al, Cancer Res 2009). Here we identify regulatory elements involved in DIAPH3 expression. Methods: The DIAPH3 promoter was cloned, chromatin immunoprecipitation assays were carried out, and transcriptome expression in PCa cells in which DIAPH3 was silenced was assessed. Key transcription factor (TF)-target interactions were identified. Results: Serial deletion of the 5'-flanking region of the DIAPH3 gene identified the region -66/+59 as the minimal promoter containing several NF-kB binding sites, as well as a repressive region in -459/-276. DIAPH3 mRNA was down-regulated by TNF-alpha, suggesting involvement of the NF-kB pathway in DIAPH3 transcriptional regulation. ChIP confirmed p65 subunit binding to the DIAPH3 promoter. Microarray analysis revealed 3,036 differential expressed genes in DIAPH3 deficient cells. Computational analysis suggests that NFAT5 and NF-kB are key transcriptional regulators. Conclusions: DIAPH3 is regulated by oncogenic signaling, including NF-kB. Further experiments are in progress to assess the role of these pathways in the transition to the amoeboid phenotype.

Outcomes for Spleen Preserving vs. Splenectomy in Distal Pancreatectomy

Marwan Sheckley, Nicholas Nissen

Introduction: Performing splenectomy alongside with distal pancreatectomy has been commonly performed. More recently, preserving the spleen has been thought of as the preferred way when treating distal pancreatic tumors. This paper is to evaluate the safety and efficacy between the two techniques performed laparascopically. Method: retrospective analysis using database and chart review for 46 patients, 22 patients underwent laparoscopic distal pancreatectomy with splenectomy (DPS) and 24 patients underwent laparoscopic spleen-preserving distal pancreatectomy (SPDP). All patients were operated on by a single surgeon during the period of 2007-2013. Patients: out of the 46 patients, there were 22 males, 24 females and mean age=64.42. Most common diagnosis were neuroendocrine tumor (NET, n=15) and adenocarcinoma (n=10). Results: For DPS patient, mean LOS= 7 days, mean blood loss 444 ml, mean duration of procedure 304 minutes, 2 patients developed infections post surgery and 1 had pancreatic leak and 1 with peripancreatic abscess For SPDP, mean LOS= 5 days, blood loss 325 ml, and mean duration of procedure 300 minutes. 1 patient with leukocytosis due to spleen infraction and 1 patient with intra abdominal abscesses Conclusion: DPS patients did not show better outcomes than patients with SPDP, and preserving the spleen should be taken into consideration when performing distal pancreatectomy surgeries.

A HER3-binding nanoparticle targets and kills Trastuzumabresistant HER2+ breast cancer

Jessica Sims, Michael Taguiam, Chris Hanson, Xiaojiang Cui, Lali Medina-Kawe

The human epidermal growth factor receptor subunit (HER2), or ErbB2, is a receptor tyrosine kinase that is amplified in approximately 20-25% of invasive breast cancers. Anti-HER2 therapies such as trastuzumab (Herceptin®) have become important in the management of these aggressive and metastatic breast cancers. Although many patients with HER2-positive breast cancer initially respond to anti-HER2 treatments a significant portion of them develop resistance to these therapies. Consequently, there is a great need to develop new drugs that are effective against these resistant tumors. Recently, it has been shown that another member of the HER family, HER3, is commonly upregulated in these drug-resistant cancers. This observation led us to develop a unique drug delivery protein, called HerPBK10, which specifically targets the cell surface receptor, HER3. HerPBK10, once it has bound to the HER3 receptor, triggers rapid endocytosis and endosomal penetration, enabling it to deliver a toxic payload to the cell, resulting in cell death. We hypothesized that cytotoxic drugs delivered by HerPBK10 would induce significant targeted cell death in trastuzumab-resistant HER2+ breast cancers due to the high levels of HER3 and would therefore provide an effective treatment for patients who have developed resistance to traditional therapies. We have demonstrated that HerPBK10 binds to the cell surface of three different HER2+ breast cancer cell lines and that this binding can becompetitively inhibited by free HER3 ligand, indicating that HerPBK10 binds specifically to HER3. Next, we showed in multiple

trastuzumab-resistant cell lines that HER3 receptor levels are significantly increased in drug-resistant cells, confirming the results of other researchers. We then assembled our targeted molecule, HerPBK10 with the chemotherapeutic doxorubicin. Theresulting nanoparticle, called HerDox, was used to treat two different HER2+ breast cancer cell lines that are susceptible to trastuzumab treatment and two that have acquired resistance to trastuzumab. We demonstrated that HerDox caused cell death in all cell lines, but at a greater level and at a lower dosage in the drug-resistant lines. We also compared the effect of the HerDox nanoparticle to trastuzumab and showed that it caused greater overall cell death. In addition, we combined our nanoparticle with trastuzumab, and showed that together, they havean additive effect on cell death. These results indicate that our HER3 targeting nanoparticle, HerDox, efficiently targets and kills cancer cells that have become resistant to trastuzumab, and has the potential to be used either as a single drug or as part of a combinatorial therapy in eliminating drug-resistant HER2+ breast cancers. We are in the process of verifying these findings in vivo in order to demonstrate the potential of HerDox as a treatment for patients who have become non-responsive to traditional anti-HER2 therapies.

A Smarter Smart-Bomb: Engineering a Novel Ad5 Penton Base for More Efficient Molecular Trafficking

Dustin Srinivas

Our novel fusion protein, HerPBK10, has provided us with a way to specifically target Her2+ cancer cells and deliver a toxic payload resulting in cancer cell death. The Penton Base or “PB” portion of this protein enables the fusion protein to escape from the endocytic system and be trafficked to different parts of the cell. In an effort to improve the targeting of our fusion protein, we used a novel technique called Directed Evolution to modify the penton base so that it more efficiently targets either the nucleus or the cytoplasm of the cell. Through the creation of a mutant penton base phage library we were able to induce selective pressure and obtain fractions of PB mutants that specifically target to the nucleus or the cytoplasm. We have begun testing the targeting ability of these mutants and the cytosolic-fraction mutant, 111c3, has shown evidence toward cytosolic trafficking with little to no protein found in the nucleus shown by microscopy. Further studies are underway to confirm this phenomenon. The remaining mutated sequences are being characterized and we will then verify their ability to specifically target our fusion protein, HerPBK10, to either the nucleus or cytoplasm of the cell.

Targeting chemo-resistance in CCNE1-amplified ovarian cancer

Barbie Taylor-Harding, Hasmik Agadjanian, Paul-Joseph Aspuria, Dong-Joo Cheon, Takako Mizuno, Sandra Orsulic, Beth Karlan, Christine Walsh, Ruprecht Wiedemeyer

The putative oncogene CCNE1 (cyclin E1) is frequently amplified in human high-grade serous ovarian cancer. CCNE1-amplified cancers tend to be wildtype for BRCA1 and BRCA2 and are associated with shorter survival and resistance to platinum-based chemotherapy. Cyclin E1 is an activating cofactor for

cyclin-dependent kinases (CDK), which stimulate cell cycle progression through phosphorylation of the retinoblastoma (RB) protein and subsequent induction of E2F transcriptional activity (cyclin E1-RB-E2F signaling). Since BRCA1 and BRCA2 are known target genes of E2F transcription factors, we hypothesize that cyclin E1 signaling actively contributes to high DNA repair capacity and chemo-resistance in a BRCA1/2-dependent manner. We predict CCNE1-amplified ovarian cancers to exhibit an "anti-BRCAness" phenotype. In two independent gene expression datasets, generated by TCGA and The Women's Cancer Program at Cedars-Sinai, we found that CCNE1-amplified ovarian cancers maintain higher levels of BRCA1 than CCNE1-wildtype tumors. Moreover, we have employed genetic and pharmacological targeting approaches in order to assess if inhibition of cyclin E1-dependent signaling can induce BRCAness and restore chemo-sensitivity. Here, we show that pharmacological CDK inhibitors (CDKi) effectively shut down E2F-mediated transcription, resulting in downregulation of BRCA1 and BRCA2 in CDKi-treated cells. Long-term exposure of ovarian cancer cell lines to CDKi selected for cells with reduced dependency on cyclin E1, and genomic profiling revealed de novo DNA copy number changes which compensate for loss of cyclin E1 function. Importantly, CDKi-resistant subclones retained lower levels of BRCA1 and were significantly more sensitive to cisplatin than parental cell lines. Collectively, our results suggest that cyclin E1 signaling is required for BRCA1 expression and chemo-resistance. Currently available CDKi such as Dinaciclib, which is in a phase 3 clinical trial, may be useful to specifically sensitize CCNE1-amplified ovarian cancers to cisplatin.

FOXC2 Expression Promotes Epithelial-to-Mesenchymal Transition and Resistance to Anoikis in Ovarian Cancer

Tom Thomas, Jiangyong Miao, Maricel Gozo, Dong-Joo Cheon, Jessica Beach, Ann Walts, Beth Karlan, Sandra Orsulic

FOXC2 is a forkhead family transcription factor that plays a critical role in specifying mesenchymal cell fate during embryogenesis. Expression of FOXC2 was recently shown to be associated with breast cancer and esophageal squamous cell carcinoma in which it is believed to promote tumor progression by epithelial-to-mesenchymal transition (EMT). However, the role that FOXC2 plays in other tumor types has yet to be elucidated. Focusing on ovarian cancer, we show that the FOXC2 protein is expressed in certain subtypes of ovarian cancer (OVCA), including clear cell carcinomas and malignant mixed Mullerian tumors (MMMT), both of which are associated with chemoresistance and poor clinical outcome. FOXC2 overexpression in murine OVCA lines induces mesenchymal-like cell morphology and enhances cell proliferation, colony formation in soft agar, and tolerance for anoikis. In vivo, FOXC2 enhances growth of subcutaneous and intraperitoneal xenograft tumors. Preliminary work in human OVCA lines also suggests EMT as a potential mode of action for FOXC2 in the progression of aggressive forms of ovarian cancer.

Foxp3+ T cells inhibit antitumor immune memory modulated by mTOR inhibition

Yanping Wang, Robert Figlin, Hyung Kim

Inhibition of mTOR signaling enhances antitumor memory lymphocytes. However, pharmacologic mTOR inhibition also enhances regulatory T cell (Treg) activity. To counter this effect, Treg control was added to mTOR inhibition in preclinical models. Tregs were controlled with CD4 depleting antibodies because CD4 depletion has high translational potential and already has an established safety profile in patients. The antitumor activity of the combination therapy was CD8 dependent and controlled growth of syngeneic tumors even when an adoptive immunotherapy was not used. Lymphocytes resulting from the combination therapy could be transferred into naive mice to inhibit aggressive growth of lung metastases. The combination therapy enhanced CD8 memory formation as determined by memory markers and functional studies of immune recall. Remove of Tregs was the mechanism underlying immune stimulation and memory formation following CD4 depletion. This was confirmed using transgenic DEREG (depletion of regulatory T cells) mice to specifically remove Tregs. It was further confirmed in reciprocal studies where adoptively transferred tumor-specific Tregs neutralized the immune stimulation of CD4 depletion. Also contributing to tumor control, Tregs that eventually recovered following CD4 depletion were less immunosuppressive. These results provide a rational for further study of mTOR inhibition and CD4 depletion in patients.

Scaffold attachment factor B1 (SAFB1) regulation of intracrine androgen

Julie Yang, Mirja Rotinen, Sungyong You, Jayoung Kim, Michael Freeman

Androgen receptor (AR) interacts with a variety of modifier proteins to regulate gene expression during prostate cancer (PCa) progression. We have identified the nuclear matrix protein SAFB1 as an AR co-repressor as well as an interacting partner and phosphorylation target of the pro-survival kinase Akt1 and the growth inhibitory kinase Mst1. Microarray data from stable SAFB1 knockdown cells indicated that SAFB1 may play a role in regulation of intracrine androgen levels in the prostate through the regulation of the UDP-glucuronyltransferase 2B (UGT2B) family of genes. This enzyme family has been shown to regulate active androgen levels through glucuronidation of androgens. This irreversible process solubilizes the hormones so that they can be exported from cells and secreted in the urine. Our microarray data showed that UGT2B 7, 10, 11, 15, 17, and 28 were significantly downregulated in LNCaP PCa cells in which SAFB1 was stably silenced. Downregulation of UGT2B11, 15, 17, and 28 was validated with real time PCR (RT-PCR). Conversely, enforced expression of SAFB1 was able to upregulate UGT2B gene family members in LNCaP cells. Because UGT2Bs play a role in regulating active androgen levels within the prostate tissue, it is hypothesized that the loss of SAFB1 may deregulate androgen homeostasis. Elucidating the potential role of SAFB1 in regulating the level and function of UDP-glucuronisyltransferases, which determine the end point of androgen signaling, will be important in understanding mechanisms of intracrine androgen signaling in castration-resistant PCa.

Comparison of two animal models for evaluating neoadjuvant and adjuvant therapies

Hao Zhang, Hyung Kim, Yanping Wang

Approximately 20% of patients with clinically localized renal cell carcinoma (RCC) have tumor recurrence after partial or radical nephrectomy. Systemic therapies that can reduce the risk of metastatic recurrence are needed. Various animal models have been proposed to evaluate novel adjuvant therapies. We used two different models of recurrent RCC following surgical removal of the primary tumor. Temsirolimus is a first generation mTOR inhibitor that has been FDA-approved for the treatment of metastatic RCC. We evaluated the efficacy of temsirolimus as adjuvant or neoadjuvant therapy with the goal of comparing results in our two models. In the first model, following surgical removal of the primary tumor, recurrence was simulated by injection of intravenous tumor cells to produce lung metastasis, and temsirolimus as adjuvant therapy was effective in inhibiting metastatic tumor growth. However, when tumor recurrence was simulated by subcutaneous injection of tumor cells in the second model, both adjuvant and neoadjuvant temsirolimus therapy promoted recurrent tumor growth. These findings underscore the limitations of animal studies for evaluating adjuvant and neoadjuvant therapy and the need for validated preclinical models.

Cardiovascular and Respiratory Disease

Acute Statin-Induced Cardioprotection Requires Parkin-Mediated Mitophagy; A Benefit Which Is Lost With Coenzyme Q10 Supplementation

Allen Andres, Gerano Hernandez, Pamela Lee, Chegnqun Huang, Eric Ratliff, Jon Sin, Christine Thornton, Marichris Damasco, Roberta Gottlieb

The mechanism of how statins acutely protect the heart against ischemic damage remains unclear. Statins inhibit HMG-CoA reductase, the rate limiting step in cholesterol synthesis. Production of coenzyme Q10 (CoQ10) is also affected which leads to diminished mitochondrial membrane potential (MMP). Loss of MMP promotes degradation of mitochondria through autophagy (mitophagy). Recently we reported that ischemic preconditioning requires Parkin-mediated mitophagy. Therefore, we hypothesized that acute cardioprotection by statins involves mitophagy. Simvastatin treated HL-1 cardiomyocytes (1μM, 24h) and cardiac tissue of mice (20mg/kg, 4h post i.p. harvest) exhibited diminished Akt/mTOR signaling and increased autophagy. Moreover, statins triggered mitochondrial fragmentation, and translocation of Parkin and p62/SQSTM1 to the mitochondria leading to increased mitophagy. Co-supplementation with CoQ10 blocks these effects. To establish the requirement for statin-mediated mitophagy in cardioprotection, we examined the ability of statins to reduce infarct size in Parkin knockout (KO) mice which are compromised in inducing cardiac mitophagy under ischemic challenge. Statin sreduced infarct size from 55% of area at risk to 30% in wild type mice, but had no protective benefit in Parkin KOs. Astonishingly, co-treatment with CoQ10 abolished statin benefit in wild-type mice. These findings suggest that statins elicit cardioprotection by promoting mitophagy via the depletion of CoQ10.

A Two Minute Test During Rounds Can Replace a Spontaneous Breathing Trial

Matthew Bloom, J Lu, T Tran, J Mirocha, M Bukur, R Chung, E Ley, N Melo, A Salim, D Margulies

Introduction: The Spontaneous Breathing Trial (SBT) is commonly used to assess for extubation readiness, but takes 30-60 minutes to administer. A two-minute test affords rapid evaluation during rounds. We hypothesized that a two-minute pre-extubation test (2MIN) could replace the SBT. The primary endpoint was ability to predict successful extubation. The secondary endpoint was missed opportunities to extubate. Methods: Data were prospectively collected on all patients endotracheally intubated for >48 hours nearing extubation in a tertiary center's mixed trauma/surgical ICU from August 2012 to August 2013. The SBT was performed for at least 30 minutes at 40% FiO2, PEEP 5, PS 8. This was followed by a 2MIN trial, in which patients were disconnected from the ventilator and directly observed. Patients who failed the SBT were allowed to recover for several hours before performing the 2MIN trial. A successful 2MIN was defined all of: heart rate < 120, systolic blood pressure 90-180, respiratory rate < 35, SpO2 > 90%, and no signs of patient agitation. The decision to extubate was made at the attending's

discretion. Successful extubation was defined as not requiring reintubation within 48 hours. Results: 128 sets of evaluations were performed, resulting in 91 extubations. 84(92.3%) of these were successful. The 2min test correctly predicted success in 75/81(92.5%) extubations vs. 82/89(92.1%) via SBT.. 9/84(10.7%) of the successful extubations were missed (1-sensitivity) by the 2MIN test vs. 2/84 (2.3%) with an SBT. No adverse effects were attributed to the 2MIN test. Oxygen saturation <90% was common in 26 (of 33) 2min failures, tachypnea in 5, hypertension in 3, and tachycardia in 3. Conclusion: The 2min test predicts extubation success with rates similar to that of the longer SBT. However, the 2min test missed more opportunities for extubation than the SBT. The most efficient liberation regiment may consist of a two minute screening test and immediate extubation for those who pass, followed by an SBT for the others. Additional studies may further improve the overall predictive accuracy of the 2min and may suggest optimization of its parameters.

Is Cold Pressor Cardiac Magnetic Resonance Imaging Testing Useful for Detection of Coronary Endothelial Dysfunction?

Sherwin Dela Cruz, Janet Wei, Chrisandra Shufelt, Puja Mehta, Andre Rogatko, Xiao Zhang, Louise Thomson, John Petersen, R. David Anderson, Carl Pepine, Daniel Berman, C. Noel Bairey Merz

Coronary endothelial dysfunction is typically diagnosed by invasive coronary reactivity testing (CRT) to measure change in coronary blood flow (ΔCBF) and diameter response to acetylcholine (Ach). We evaluated if cold pressor testing (CPT) during noninvasive cardiac magnetic resonance imaging (CMRI) measurement of myocardial perfusion reserve index (MPRI) is associated with invasive measures to Ach and CPT. CRT with Ach and CPT was conducted as previously published. Normal ΔCBF defined as > 50%. Normal diameter response was dilation > 0%. CPT CMRI (1.5 T) measured MPRI of the whole heart and subendocardial region. Wilcoxon Scores were used for analysis to compare MPRI in women with normal and abnormal invasive ΔCBF and diameter response to CPT or Ach. Polychoric correlations were also performed for all MPRI measurements. CPT MPRI was not significantly associated with invasive CPT and Ach measurements (Table). There were no correlations noted for all variables tested (all p values > 0.05). CPT MPRI determined with CMRI was not different between those with normal and abnormal response to CPT and Ach during invasive testing. These data suggest that CPT during CMRI may not be useful for detection of endothelial dysfunction noninvasively. Additional investigation will evaluate CPT CMRI and cardiovascular outcomes.

Vascular endothelial function in Mongolian women with a history of preeclampsia or gestational hypertension

Enkhmaa Davaasambuu, Chrisandra Shufelt, Janet Rich-Edwards, C. Noel Bairey Merz

Preeclampsia, characterized by new onset hypertension and proteinuria, occurs 5% in US and up to 15% of pregnancies in Mongolia. Vascular endothelial dysfunction has been implicated in the pathogenesis of

both preeclampsia and CVD, but less is known about gestational hypertension. We aimed at assessing vascular function in postpartum women with a history of preeclampsia, gestational hypertension or normotensive pregnancy in Mongolia. Charts of women admitted for preeclampsia, gestational hypertension, or normotensive pregnancies were reviewed retrospectively. 38 women at 8 to 24 months postpartum, with a history of preeclampsia (n=14), gestational hypertension (n=11), or normotensive pregnancy (n=13) agreed to participate. Postpartum arterial stiffness was assessed by augmentation index adjusted for heart rate of 75 bpm (AIx75) and pulse wave velocity (PWV). Statistical analysis was performed by one-way ANOVA. Women with preeclampsia or gestational hypertension had a higher AIx75 compared to normotensives (23.2±7.4%, 25.1±8.6%, 14.1±12.5%, respectively; $p<0.01$). In contrast, there was no significant difference in PWV (7.84±1.4m/s, 7.43 ±1.0m/s, 7.07 ±1.5m/s, respectively; $p=0.35$). Our data are consistent with other published research that reports impaired endothelial function after preeclampsia or gestational hypertension compared to normal. Our ongoing work is focused on vascular function measurement prior to, during and after pregnancy.

Cardiac BIN1 Folds T-tubule Membrane, Controlling Ion Flux and Limiting Arrhythmia

TingTing Hong, Huanghe Yang, Shan-Shan Zhang, Michael Grabe, Lily Jan, Robin Shaw

Cardiomyocyte T-tubules are important for ion channel regulation. Bridging Integrator 1 (BIN1), a membrane scaffolding protein associated with calcium channel trafficking, is down-regulated in failing hearts. Here we find that BIN1 causes dense microscopic folding of cardiac T-tubule membrane. In mice, heterozygous cardiac specific Bin1 deletion does not affect gross cardiomyocyte morphology, but on detailed microscopy and electrophysiology studies we find less T-tubule folding. Less folding increases diffusion of local extracellular calcium and potassium ions, prolonging action potential duration, and resulting in increased ventricular ectopy. We also find that T-tubule folds are rescued by a particular BIN1 isoform (BIN1heart), that occurs in heart but yet not skeletal muscle, and that BIN1heart uses actin to stabilize T-tubule membrane at Z-discs. In conclusion, BIN1heart recruits the actin cytoskeleton to fold T-tubule membrane, creating a fuzzy space that protectively restricts ion flux. When BIN1 is decreased in heart failure, arrhythmias can result.

A cardiac BIN1 isoform is detectable in plasma and reduced in human heart failure

Mariya Kalashnikova, Robin Shaw, TingTing Hong

Bridging Integrator 1 (BIN1) is a membrane scaffolding protein that targets L-type calcium channels to the cardiac t-tubule membrane. BIN1 expression is reduced in failing human cardiomyocytes, resulting in impaired L-type calcium channel trafficking and abnormal calcium transients. Strikingly, BIN1 has been identified in the plasma and is correlated with the incidence of heart failure and arrhythmias in patients with arrhythmogenic right ventricular cardiomyopathy, thus showing promise as a novel cardiac biomarker. However, as BIN1 is also abundant in skeletal muscle, it is vital to establish the origin of

plasma BIN1. Using PCR analysis of cDNA from human tissue, we characterize the pattern of BIN1 alternative splicing in both human heart and skeletal muscle. Similar to mouse tissue, a particular splice variant of BIN1 (BIN1Heart) with inclusion of exon 13 is expressed prominently in human heart but not skeletal muscle. Western blot and immunoprecipitation assays further confirm the expression of the protein isoform (BIN1Heart) encoded by this particular splice variant. Interestingly, immunoprecipitation of BIN1 proteins from human plasma reveals a plasma BIN1 protein profile similar to cardiac muscle but not skeletal muscle. In heart failure, expression of BIN1Heart is further decreased than ubiquitous BIN1 in both heart and plasma samples from the same patient. In conclusion, we have identified a cardiac prominent BIN1 isoform (BIN1Heart), which can be released into circulation, is reduced in heart and plasma from patients with heart failure. Plasma BIN1Heart is a potential cardiac specific biomarker for human heart failure.

Inositol-1,4,5-Trisphosphate-Mediated Spontaneous Activity in Mouse Sinoatrial Nodal Cells

Nidhi Kapoor, Rui Zhang, Jeanney Kang, Kirstin Cook, Joshua Goldhaber

Sinoatrial node (SAN) automaticity is due to the interplay of several membrane currents, including the current produced by Na-Ca exchanger (NCX) in response to Ca cycling. Several lines of evidence suggest that inositol-1,4,5-triphosphate receptors (IP3Rs) that mobilize [Ca]i are implicated in generation of spontaneous activity in embryonic and adult cardiomyocytes. However, whether IP3 signaling influences automaticity in the SAN is still controversial. To address this, we used NCX KO SAN cells to study IP3 signaling in a system of Ca cycling where Ca flux across the plasmalemmal membrane is significantly decreased. We recorded spontaneous Ca oscillations in WT and KO SAN cells in the presence of an IP3R blocker or during inhibition of phospholipase C (PLC). We found that superfusion with the IP3R blocker, 2-APB or PLC antagonist, U73122 decreased the frequency of Ca oscillations. Alternatively, an increase in IP3 production using the α-1 adrenergic receptor agonist phenylephrine led to an increase in the frequency of Ca oscillations. In summary our results indicate that Ca release from IP3Rs can modulate Ca oscillation frequency in SAN cells and support our hypothesis that IP3 signaling modulates the Ca cycling processes that regulate pacemaker frequency in the SAN.

Urine Albumin-Creatinine Ratio is Related to Endothelial Dependent Coronary Microvascular Dysfunction: A Report From the Women's Ischemia Syndrome Evaluation – Coronary Vascular Dysfunction (WISE-CVD) Study

Sofy Landes, Janet Wei, Puja Mehta, Chrisandra Shufelt, Margo Minissian, Carl Pepine, Eileen Handberg, Xiao Zhang, Andre Rogatko, George Sopko, C. Noel Bairey Merz

Urine albumin-creatinine ratio (UACR) is a measure of renal microvascular dysfunction and predicts clinical cardiovascular events. Coronary microvascular disease (CMD) is prevalent in women with ischemia but non-obstructive coronary artery disease (CAD) and is diagnosed by invasive coronary

reactivity testing (CRT). We hypothesize UACR can be a marker for predicting CMD. We measured UACR in 74 women with suspected CMD and non-obstructive CAD who underwent CRT. Left ventricular end-diastolic pressure (LVEDP) at rest and left anterior descending coronary artery diameter change to intracoronary acetylcholine and cold pressor testing (endothelial dependent functions) were obtained. Univariate linear regression was used to evaluate log UACR and CMD parameters. Women were mean age 53±11 years with BMI 30±9 kg/m2, Caucasian (64%), hypertensive (42%), current or former smokers (41%), diabetic (14%) and dyslipidemic (8%). ACE-inhibitor or angiotensin-receptor blocker use was present in 23%. Mean UACR was 18±59 mg/g, and mean LVEDP was 15 ± 5 mmHg. Coronary artery diameter responses to acetylcholine and to cold pressor had inverse relationships with UACR. LVEDP had a trend towards significance. UACR is inversely related to invasive parameters of coronary endothelial dysfunction determined by acetylcholine and cold pressor testing. This suggests a relationship between CMD and renal microvascular dysfunction.

Immunopathology of Kawasaki Disease Vasculitis Mouse Model. Role of CD11c+ Dendritic Cells, CD8 T-cells and MyD88 signaling in both hematopoietic and stromal cells

Youngho Lee, Daiko Wakita, Norika Chiba, Kenichi Shimada, Shuang Chen, Timothy Crother, Michael Fishbein, Thomas Lehman, Moshe Arditi

Background - Kawasaki disease (KD) is the most common cause of acute vasculitis and acquired cardiac disease among US children. In a mouse model of KD we have shown that TLR2/MyD88 signaling and presence of T cells are required for this KD vasculitis mouse model. However, the role of Dendritic Cells (DCs), and the specific T-cell subsets, and the cellular origins for the MyD88 signaling that are critically involved in this model are unknown. Methods and Results. We investigated the role of T-cell subsets, including CD8, CD4, and NK-T using specific gene deletion in mice or antibody depletion approaches in our KD model. We also investigated the role of CD11c+ DCs, as well as the role of MyD88 signaling in hematopoietic versus stromal cells in this mouse model of vasculitis, using bone marrow chimera experiments. Conclusions - Our results suggest that CD11c+ DCs are essential to the development of coronary lesions in our KD mouse model, and that CD8 T cells, but not CD4, or NKT cells contribute to the formation of coronary lesions. MyD88 signaling was important in both the hematopoietic and stromal cell compartments, underlying the complexity of this disease model and the involvement of both TLR and IL-1 signaling.

Wnt Signaling Promotes Pacemaker Myocyte Specification of Differentiating Cardiac Progenitor Cells

Wenbin Liang, Elizabeth Kim, Jordan Mak, Eduardo Marban, Hee Cheol Cho

Embryonic stem cells (ESCs) can give rise to cardiomyocytes, but mechanisms of cardiac subtype specification to either pacemaker cells or chamber (atrial/ventricular) cardiomyocytes remain little understood. Canonical Wnt signaling plays a key role for stem cell maintenance, and becomes

inactivated during differentiation. We hypothesized that control of Wnt signaling by Dkk1, an endogenous secreted protein inhibitor of canonical Wnt signaling, may impact cardiac subtype specification during ESC differentiation. Mouse ESCs were treated with activin-A and BMP-4 for 40 hours to initiate cardiac differentiation. At day-4, Flk-1+/PdgfR-α + cardiac progenitors were FACS-purified and seeded as monolayers. The monolayers were either treated with a saturating level of exogenous Dkk1 ("exo-Dkk1" , 150 ng/ml), or cultured in the endogenous level of Dkk1 ("endo-Dkk1" , 3.2 ng/ml as determined by ELISA). At day-8, 65% of cells were positive for cTnT, a pan-cardiac myocyte marker, with no discernible difference between endo- and exo-Dkk1 groups. At day-8, endo-Dkk1 group exhibited significantly higher levels of cardiac pacemaker cell markers (Tbx18 and Shox2), but lower levels of chamber lineage markers (Nkx2.5, Isl1, Scn5a and Cacna1c, $p<0.01$). At day-10, endo-Dkk1 group began to beat faster compared to exo-Dkk1 monolayers, and the superior automaticity became more accentuated upon further differentiation (161.5±11.5 vs. 48.0±2.9 bpm, endo- vs. exo-Dkk1, $p<0.01$, week-3). Endo-Dkk1 monolayers yielded more cells with spontaneous intracellular calcium oscillations compared to exo-Dkk1. Single, spontaneously-beating cells isolated from the endo-Dkk1 monolayers were frequently spindle-shaped, exhibited robust HCN4 proteins and I(f) currents, and fired rhythmic action potentials (288±51 bpm, n=5). Analogous results were attained with two other endogenous Wnt antagonists, Sfrp1 and Sfrp5. Our data demonstrate that inhibition of Wnt signaling promotes cardiac differentiation of ESCs, and the endogenous pathway defaults to cardiac pacemaker cells rather than chamber cardiomyocytes. Our findings provide a control point from which one could enrich either pacemaker cell or chamber myocyte population.

Burden of Sports-Related Sudden Cardiac Death in a US Community

Eloi Marijon, Audrey Evanado, Carmen Teodorescu, Kyndaron Reinier, Kumar Narayanan, Adriana Huertas-Vazquez, Katherine Jerger, Ronald Mariani, Eric Stecker, Harpriya Chugh, Jo Navarro, Jonathan Jui, Sumeet Chugh

Introduction: There is no information regarding the burden of sports-related sudden cardiac death (SCD) in the US general population. Methods: SCD cases aged 15-75 yrs were identified in a large, ongoing, prospective, population-based study in a Northwestern US metro region (population approx. 1 million) (2002-2012). Results: Of the 1894 SCD cases, 82 (4.3%) occurred during sports, yielding an incidence of 14.7 (95%CI 7.6-21.8) per million per year. The incidence of sport-related SCD was higher among men compared to women, especially in the 35-54 year age group (RR 10.66, 95% CI 1.37-82.57). Cases of sports-related SCD, compared to those unrelated to sports, were younger (50.6±12 vs. 55.9±14 years, $P<0.0001$), more likely to be male (92.7 vs. 72.0 %, with more one cardiovascular risk factor (41.0 vs. 62.1 %, $P<0.0001$), greater proportion witnessed (88.6 vs. 54.2%, $P<0.0001$), with more bystander cardiopulmonary resuscitation (50.6 vs. 25.6%, $P<0.0001$), and greater proportion of ventricular fibrillation (85.9 vs. 49.3%, $P<0.0001$). Survival to hospital discharge was higher for sports-related SCD (25.3 vs. 12.8 %, $P=0.01$).Conclusions: SCD during sports has distinct characteristics and outcome compared to overall SCD, with women appearing to have a particularly low risk.

Left Ventricular Diameter Adds Prognostic Value to Ejection Fraction in Risk Stratification for Sudden Cardiac Death

Kumar Narayanan, Kyndaron Reinier, Carmen Teodorescu, Audrey Uy-Evanado, Harpriya Chugh, Gregory Nichols, Karen Gunson, Jonathan Jui, Sumeet Chugh

Introduction: Current approaches to risk stratification for sudden cardiac death (SCD) rely almost exclusively on the left ventricular (LV) ejection fraction (EF). LV size is routinely measured in the echocardiogram but has not been considered in risk assessment.Hypothesis: We assessed the hypothesis that consideration of LV diameter improves SCD risk assessment in those with low EF.Methods: From a large ongoing, population-based study of SCD in a metro region of the Northwestern US (population approximately 1 million), SCD cases age $\geq$18 years with echocardiograms available prior (but unrelated) to the SCD event were compared with population-based controls. Severe LV dysfunction (LVD) was defined as EF $\leq$35%. LV size, measured using the LV internal dimension in diastole (LVIDD), was categorized as normal, mild, moderate or severe dilatation using American Society of Echocardiography (ASE) recommended cut-offs. Case-control comparisons were performed using the t-test or chi-square test and multiple logistic regression.Results: Case subjects (n=418) were slightly older than controls (n=329) (69.5 ± 13.8 vs. 67.7 ± 11.9; p=0.06), were more likely to be African American (11.8% vs 4.2%; $p < 0.001$), and more commonly had severe LVD (30.5 % vs. 18.8%; p=0.001). The mean LV size (52.2 ± 10.5 mm vs. 49.7 ± 7.9 mm; $p < 0.001$) and LV size index adjusted for body surface area (BSA) (26.6 ± 5.3 mm/m2 vs. 25.4 ± 4.2 mm/m2; p = 0.001) were significantly higher in cases. Moderate or severe LV dilatation (16.3% vs. 8.2%; p = 0.001) and severe LV dilatation (8.1% vs. 2.1%; $p < 0.001$) were significantly more likely to be observed in cases. In multivariable logistic regression, severe LV dilatation was an independent predictor of SCD (OR 2.5; 95% CI 1.03-5.9; p=0.04), adjusting for age, black race and low EF. Subjects with both low EF and severe LV dilatation had an OR for SCD of 3.8 (1.5-10.2) while for those with only low EF, the OR was 1.7 (1.2-2.5) suggesting that severe LV dilatation additively increased the risk of SCD.Conclusions: Increased LV diameter adds incremental prognostic value to LVEF in risk prediction for SCD in the community; this readily available echocardiographic measure warrants further evaluation.

The pH sensor of the cardiac Na+-Ca2+ exchanger

Michela Ottolia, John Scott, Joshua Goldhaber

Myocardial ischemia is a leading cause of morbidity and mortality in developed countries. The detrimental effects of ischemia are in large part due to the accompanying cellular acidosis. At the myocyte level, an increase in H+ causes aberrations in intracellular Ca2+ handling, thereby affecting contractility. Among the proteins regulated by cytoplasmic H+ is the cardiac Na+-Ca2+ exchanger (NCX), a plasma membrane transporter which extrudes Ca2+ on a beat to beat basis. Inhibition of NCX by cytoplasmic H+ could lead to contractile dysfunction. Remarkably there is little information about the

modulatory mechanisms of H+ on NCX activity. To test the hypothesis that NCX histidines are fundamental to proton inhibition of NCX activity we combined mutagenesis and electrophysiology. Our data reveal that among the 18 histidines found in NCX, four play an important role in its regulation by protons. Replacement of these residues with alanine abolished proton inhibition of NCX. Moreover we extended these findings by demonstrating that the two Ca2+ binding domains play little role in pH sensitivity: NCX mutants incapable of binding Ca2+ were still sensitive to pH. These findings suggest that NCX possesses a true, structurally distinct pH sensor which does not involve the Ca2+ binding site.

Autoregulation of connexin43 gap junction formation by internally translated isoforms

James Smyth, Robin Shaw

During each heartbeat, cell-cell electrical coupling via connexin 43 (Cx43) gap junctions allows billions of individual cardiomyocytes to synchronously contract. The Cx43 protein turns over rapidly, rendering regulation of Cx43 trafficking a critical and continuous cellular need. Altered Cx43 trafficking during heart disease disrupts intercellular coupling and can contribute to the arrhythmias of sudden cardiac death. Better understanding of how Cx43 channels are transported to the cell surface will lead to therapies that can preserve normal electrical coupling of diseased heart muscle. We have identified a novel regulatory mechanism whereby internal translation of the coding sequence of Cx43 (GJA1) mRNA generates N-terminally truncated isoforms that regulate trafficking of full length Cx43 channels. Biochemical analysis of human heart and cell lines reveals at least four additional Cx43 isoforms, with a 20 kDa isoform predominating. We find that in frame AUG codons within GJA1 mRNA represent isoform translation initiation sites and that their ablation arrests trafficking of full-length Cx43. The predominant 20 kDa isoform is sufficient to rescue this trafficking defect when expressed in trans, implicating an essential role as a trafficking chaperone for Cx43. Consistent with these data, expression of Cx43 truncated isoforms is enhanced through inhibition of the PI3K/AKT/mTOR pathway in cardiomyocytes, and concomitantly increases Cx43 gap junction plaque size. Such cap-independent internal translation initiation events may represent a common mechanism for auto-regulation of membrane protein trafficking which is likely altered during stress and, based on these studies, represents a potent target for therapeutic preservation of intercellular coupling in heart disease.

A micropatterning approach for unlocking cytoskeletal regulation of Cx43 trafficking to defined cell-cell junctions

Shan-Shan Zhang, Robin Shaw

With each heartbeat, millions of cardiomyocytes work together to propagate electrical excitation and generate the contractile force needed to circulate blood. Essential to cardiac excitation in the ventricular pumping chambers is the precise expression and timely delivery of connexin 43 (Cx43) proteins that couple neighboring cardiomyocytes. Growing evidence supports a microtubule-based trafficking

paradigm for Cx delivery directly to adherens junction structures at the ventricular intercalated disc (ID).Recently, noncontractile actin has also been implicated in Cx43 localization to the ID. A limitation of trafficking assays involving cultured cells, in which cell-cell contacts are important, is the inability to control for cell geometry or reproducibly generate points of cell-cell contact. Here we present a micropatterning-based cell pairing system that is well suited for examining how the microtubule and actin cytoskeleton confer specificity to Cx43 forward trafficking to a precisely defined cell-cell junction.

Coxsackievirus B3 Induces Premature Differentiation of Cardiac Stem Cells by Upregulating Autophagy

Jon Sin, Roberta Gottlieb, Ralph Feuer

Coxsackievirus B (CVB) is an enterovirus that most commonly causes a self-limited febrile illness in young children, but in rare severe cases can progress to myocarditis, pancreatitis, or meningo-encephalitis. Long-term consequences of mild CVB infection are unknown, however CVB antibodies are encountered more frequently in patients with idiopathic heart failure than in the general population, raising the possibility that these infections could manifest in severe chronic cardiac damage, despite showing no acute symptoms. CVB type 3 (CVB3) has previously been documented to activate host autophagic machinery upon infection, and we show here that CVB3 infection of the heart triggers a significant upregulation of autophagy in cardiac stem cells (CSCs). We have previously observed that autophagy is a crucial process for cellular differentiation, and we demonstrate now that this infection-mediated upregulation of autophagy causes premature differentiation of CSCs in the neonatal heart. This phenomenon leads to an exhaustion of CSCs that is sustained through adulthood. Though neonatally-infected adult animals appear phenotypically normal with no cardiac abnormalities, exercise or pharmacologically-induced cardiac stress triggers a significant heart failure response, suggesting a link between early stem cell depletion and a sensitivity towards late-onset heart failure.

Burst Behavior of Calcium Transients in the Sinoatrial Node of Sodium-Calcium Exchange Knockout Mice

Angelo Torrente, Audrey Zaini, Ashley Rosenberg, Rui Zhang, Jeanney Kang, Kenneth Philipson, Joshua Goldhaber

Na+-Ca2+ exchange (NCX) is an acknowledged component of sinoatrial node (SAN) pacemaker activity, but its role is controversial. To explore its importance in SAN activity, we generated an atrial-specific NCX KO mouse that lives into adulthood despite a junctional rhythm and absence of atrial activity. Although irregular and disorganized, spontaneous depolarizations persist in the KO SAN, but they do not reliably spread to the atria. In this study, we examined whether abnormal intracellular calcium (Ca) release in the KO SAN might explain the unusual pacemaker activity. We used an ex vivo tissue preparation including SAN and atria, loaded with the Ca indicator Cal 520, to record cellular Ca movements using high speed 2D confocal imaging. At 20oC, Ca transients were regular in WT SAN (86±6

bpm), but slow, irregular and characterized by bursts in KO (47±14 bpm). Between these bursts of Ca transients we observed numerous intracellular Ca waves suggestive of Ca overload. At 36oC, rates were faster in both genotypes (302±14 bpm versus 129±12 bpm, respectively). Nevertheless, the burst phenotype and the generation of copious Ca waves in-between was maintained in NCX KO. We conclude that Ca overload causes the abnormal pacemaker activity found in NCX KO.

Serial Cardiac Magnetic Resonance Imaging in Women with Persistent Chest Pain: A Report From the Women's Ischemia Syndrome Evaluation-Coronary Vascular Dysfunction (WISE-CVD) Study

Janet Wei, Louise Thomson, John Petersen, Puja Mehta, Xiao Zhang, Andre Rogatko, George Sopko, Carl Pepine, Eileen Handberg, Daniel Berman, C. Noel Bairey Merz

Background: Women with persistent chest pain, evidence of ischemia and no obstructive coronary artery disease have an increased risk of major adverse cardiovascular events compared to asymptomatic women. Changes over time in left ventricular (LV) mass, mass-to-volume ratio, diastolic filling parameters, and myocardial perfusion reserve index (MPRI) have not been previously described. Methods: 134 women underwent stress cardiac magnetic resonance imaging (CMRI) at baseline and at one-year follow-up using the same pharmacologic stress agent (adenosine or regadenoson). CAAS MRV-3.3 software (Pie Medical Imaging B.V., Netherlands) was used to measure MPRI, LV mass, LV volume, ejection fraction, peak filling rate, and time to peak filling rate. Paired t-tests were performed for statistical analysis. Results: At baseline women were mean age 54 ± 11 years with body mass index 30 ± 8, Caucasian (68%), prior or current smoker (43%), history of hypertension (35%), dyslipidemia (17%), and diabetes (10%). MPRI significantly improved at the one-year follow-up visit, but there were no significant changes in the other CMRI parameters. Conclusion: Women undergoing repeat stress CMRI showed improvement in their MPRI at one-year follow-up. Further evaluation is needed to assess relations between MPRI improvement and risk factor and angina management, as well as clinical outcomes.

Endocrinology/Metabolism/Diabetes/Obesity

Selective cyclin E suppression leads to POMC gene regulation

Takako Araki, Ning-Ai Liu, Yukiko Tone, Daniel Cuevas, Anat Ben-Shlomo, Song-Guang Ren, Jian Hong, Roy Heltsley, Masahide Tone, Shlomo Melmed

Proopiomelanocortine (POMC) is the gene responsible for production of Adrenocorticotropic hormone (ACTH). Cushing disease is caused by excessive ACTH secretion from pituitary corticotroph tumors, and is associated with high morbidity and mortality. Surgical resection is the first therapy, however, the high recurrence rates make the disease challenging to cure, while pharmacotherapy is not well established. POMC is also expressed by non-pituitary sources. Ectopic Cushing syndrome is the rare neuroendocrine tumor (NET) arising from a non-pituitary origin which can cause rapid onset of hypercortisolemia and has poor prognosis. R-roscovitine is a selective CKD inhibitor (CDK1/2) to control cell cycle by inactivating cyclin/CDK complexes. Here, we found that R-roscovitine suppresses ACTH expression in both pituitary and ectopic ACTH producing tumors. We also show for the first time that R-roscovitine downregulated human POMC promoter activity through a transcription factor, E2F1. We have identified R-roscovitne response elements in the human POMC promoter, and demonstrated that E2F1 binds to the elements and the E2F1-mediated promoter activity is downmodulated by R-roscovitne. R-roscovitine is therefore a compelling candidate for medical therapy of pituitary Cushing disease and it could be the first cell targeted treatment for ectopic Cushing syndrome.

TEX264: a novel interaction partner of Lmf1, a lipase chaperone involved in plasma lipid metabolism

Candy Bedoya, Peterfy Miklos

Heart disease is the leading cause of death in the U.S; risk factors include obesity, hypertension, and hyperlipidemia. Lipoprotein lipase (LPL) is the enzyme responsible for the clearance of triglycerides (TG) and is associated with variations in plasma TG. Lipase maturation factor 1 (Lmf1) is a membrane bound protein that is found in the endoplasmic reticulum (ER) which acts as a chaperone aiding in the posttranslational modification of LPL. While Lmf1's primary role is of a lipase chaperone, we wanted to discover other potential interaction partners in order to continue to define Lmf1's role within the cell. Using a yeast two-hybrid screen we were able to identify binding of a hereto unknown protein, testis expressed protein 264 (TEX264), to Lmf1. We confirmed this finding using co-immunoprecipitation and were able to narrow down the interaction domain using systematic c-terminal truncations. Owing to the fact that little is known about TEX264 we ran prediction software whose results hinted at TEX264 being a membrane protein. Using immunohistochemistry we were able to see that TEX264 co-localizes with Lmf1 in the ER. The discovery of this novel interaction may help further elucidate Lmf1's role in the cell as well as uncover the function of TEX264.

Inhibition of nocturnal free fatty acid suppresses hyperinsulinemic compensation during diet-induced insulin resistance

Josiane Broussard, Cathryn Kolka, Ana Castro, Isaac Asare, Stella Kim, Richard Bergman

Compensatory hyperinsulinemia due to increased β-cell function and reduced insulin clearance is a normal consequence of increased caloric intake and insulin resistance. Failure of compensatory mechanisms plays a central role in pathogenesis of diabetes, therefore, it is critical to identify in vivo signal(s) involved in compensation. In an effort toelucidate these signals, studies have ruled out glycemia, cortisol, GLP-1 and growth hormone. We hypothesize that elevated nocturnal FFA are a signal for β-cell compensation during high fat feeding. Twenty-four hour blood sampling was conducted in eight male dogs at baseline (CON), after 6 weeks of fat feeding (FAT), and after fat feeding with nocturnal FFA suppression (FATsup). Suppression was achieved by injections of adenosine agonist GS-9667 (Gilead, Inc). Immediately following each 24-hr sampling period, an intravenous glucose tolerance test (IVGTT) was performed. Food was presented from 9-10am. CON diet composition was 40% carbohydrate, 28% protein, and 32% fat. During fat feeding, 6g/Kg of lard was added to achieve 52% fat diet. Fat feeding increased weight [CON 27.7(1.2); FAT 28.7(1.4); FATsup 29.0(1.3) kg; p=0.02. Post-hoc tests indicate an increase in weight after fat feeding, but no difference between FAT and FATsup]. Average fasting and nocturnal glucose and insulin levels were unchanged. Fat feeding increased nocturnal FFA, which was returned to CON levels with drug administration [CON 5.9(0.6); FAT 7.9(0.6); FATsup 5.1(0.4) mM.hr; p<0.01. Post-hoc tests indicate an increase in FFA after fat feeding, whereas CON and FATsup were not different]. Importantly, there was a strong and significant correlation between nocturnal FFA levels and AIRg [r=0.56, n=21, p<0.01]. Thus, hypercaloric diet-induced β-cell compensation was prevented when the nocturnal FFA pattern was normalized. These studies indicate that nocturnal plasma FFA is an important signal for β-cell compensation during fat feeding.

A novel structural and functional acromegaly classification

Daniel Cuevas-Ramos, John Carmichael, Odelia Cooper, Vivien Bonert, Arkadiusz Gertych, Adam Mamelak, Shlomo Melmed

Objective: To rigorously classify an acromegaly patient cohort defined by clinical, radiological, histopathological characteristics.Methods: Subjects were selected from a pituitary tumor research registry that includes 338 acromegaly patients. Results: When all patients were subjected to cluster analysis, 132 were rigorously classified to three acromegaly types. Type 1 (48%) comprised older patients with the longest follow-up and most favorable outcomes, characterized by densely granulated, non-aggressive microadenomas and macroadenomas. Type 1 tumors extend to the sphenoid sinus more frequently than suprasellar extension (concave tumor image), and express abundant immunoreactive p21 and SSTR2. Type 2 (24%) comprised non-invasive densely or sparsely granulated macroadenomas, without significant extension (flat tumor image), with intermediate biochemical outcome. Type 3 (28%), was characterized by sparsely granulated aggressive macroadenomas, and comprised patients with adverse therapeutic outcomes, despite receiving more treatments. These tumors extend to both the

sphenoid sinus and suprasellar regions with commonly encountered optic chiasm compression ("peanut" MRI image), with low tumor p21 and SSTR2 expression.Conclusions: Three acromegaly types accurately identify patients with distinctive structural and functional disease aggressiveness and outcomes. This classification is useful for clinical practice, and provides an accurate tool for clinical study selection criteria.

Portal vein sensors mediate counterregulatory response to hypoglycemia, but not exenatide's effect on oral glucose tolerance

Viorica Ionut, Orison Woolcott, Darko Stefanovski, Malini Iyer, Josiane Broussard, Hasmik Mkrtchyan, Miguel Burch, Richard Bergman

The hepato-portal area is an important metabolic sensor involved in glucose homeostasis. Low glucose is sensed in the portal vein (PV), and PV denervation blunts the counterregulatory response to hypoglycemia. PV might also be involved in sensing of hyperglycemia and of hormones such as glucagon-like peptide-1 (GLP-1). We have shown that activation of hepato-portal sensors via intraportal infusion of glucose and GLP- results in increased glucose disappearance, independent of corresponding changes in pancreatic hormones. Moreover, infusion of intraportal glucose and subcutaneous administration of a GLP-1 mimetic (exenatide) during a clamp resulted in increased hepatic glucose uptake. The aim of the current study was to investigate directly whether glucose and GLP-1 receptors residing in the PV mediate exenatide's effect to enhance liver uptake during hyperglycemia, by using portal vein denervation.PV denervation was accomplished in canines (n=7) by surgical stripping and chemoneurolysis with phenol. Denervation of PV was confirmed by reduced tyrosine hydroxylase staining and decreased PV catecholamine content.Before and after PV denervation each animal underwent: 1) hypoglycemic clamps (50 mg/dl) and 2) paired oral glucose tolerance tests (OGTT), with and without exenatide (20 mcg, sc).Catecholamine response to hypoglycemia was severely suppressed after denervation: a 90% reduction from 640+- 192 pM before, to 80+-125 pM after denervation (p=0.03).In contrast, PV denervation did not impair exenatide's effect on oral glucose tolerance (defined as the decrease in OGTT plasma glucose AUC in the presence of exenatide). Exenatide effect on AUC was 479 +-420 mg/dl *min before, and 730 +-572 mg/dl *min after denervation (p=0.66)Our data suggests that PV sensors are involved in hypoglycemic counterregulation, but do not mediate exenatide's effect on oral glucose tolerance. It is possible that receptors in the liver itself, not in the PV mediate the insulin-independent decrease in glycemia observed with intraportal administration of glucose and GLP-1 or exenatide.

Six Weeks High Fat Diet Does Not Reduce Access of Insulin to Skeletal Muscle

Cathryn Kolka, Josiane Broussard, Ana Castro, Richard Bergman

Insulin access to skeletal muscle is impaired in obesity, and we have shown that diet-induced insulin resistance reduces dispersion of insulin through skeletal muscle. Thus, we expected that a reduced amount of insulin would be detected in the interstitial fluid of the obese canine. Anesthetized dogs were exposed to basal insulin levels for 180min followed by hyperinsulinemia (1mU/min/kg); glucose was infused at a variable rate to maintain euglycemia. Fat feeding reduced the glucose infusion rate and leg glucose uptake, indicating insulin resistance. At the end of the clamp, arterial levels were 80.6+10.4mU/L in lean animals, compared to 93.0+5.0 in obese animals (n=8 per group), while lymph (interstitial) insulin concentrations were 63.4+11.5 and 55.0+4.4mU/L in lean and obese respectively. Thus, at steady state, the ratio of lymph:plasma insulin showed no significant impairment in insulin access to the interstitial space after six weeks of fat feeding, yet cellular insulin sensitivity was decreased. Therefore we conclude that insulin resistance induced by six weeks of a high fat diet is likely due to cellular insulin resistance, rather than a defect in insulin access. Further experiments are required to assess insulin access under lower insulin concentrations, or in animals with more severe insulin resistance.

Vitamin D Status and Bone Health: Quantifying the Difference between Deficiency, Insufficiency and Sufficiency

Melodie Metzger, Mark Svet, Linda Kanim, Rick Delamarter

Introduction: The desire to achieve sufficient levels of vitamin D has increased over the past decade; yet no clear standard has been set to define vitamin D deficiency, insufficiency, and sufficiency. Therefore the purpose of this study was to investigate the relationship between vitamin D dosage and bone quality, quantity and strength to better define bone health as a function of circulating vitamin D. Methods: 48 male rats were randomized into four experimentally controlled vitamin-D diets: 0 IU/g, 2.25 IU/g, 5 IU/g, and 40 IU/g vitamin D. After 16 weeks of diet manipulation, rat femora were evaluated using microCT, biomechanically, and micro-indentation procedures. Results: Femur stiffness and max loading strength were correlated to vitamin D dietary levels ($p<0.05$). Factors related to bone quantity were also significantly positively correlated with Vitamin D intake ($p<0.05$). Micro-indentation testing indicated no significant difference in local cortical shell properties between groups. Discussion: Our results indicate a trend of increasing whole-bone strength with increasing levels of vitamin D. The lack of differentiation between groups in both BMD and micro-indentation data suggests that localized bone properties may remain consistent and that the mechanism of bone fragility due to vitamin D insufficiency may lie in structural factors.

De novo diabetes after two types of pancreatic surgery: whipples and distals

Binh Nguyen, Nicholas Nissen, Run Yu

Pancreatic resection is accompanied by the risk of developing diabetes. Methods: Medical records from 138 patients who underwent a Whipple procedure and 42 patients who underwent a distal pancreatectomy, all performed by Dr. Nicholas Nissen at Cedars-Sinai Medical Center within the last six years, were retrospectively examined. Diabetes following surgery was established by physician diagnoses from the Web-VS system, hemoglobin A1C percentages (>6.4), and diabetic medications prescribed. Results: Approximately 17% (17/103) of whipple patients developed diabetes post-operation after accounting for existing diabetes prior to surgery, while 29% (10/34) of distal patients became diabetic. Average time from surgery to first documented instance of post-op diabetes for whipples was 1 year, 5 months, as opposed to 2 years, 3 months for distals. Conclusions: The incidences reported here are slightly higher than in the literature. Information such as whether the spleen was preserved, BMI, age, and history of pancreatic cancer, among others, will be analyzed in combination with radiology data consisting of islet density, tissue dimensions, and fat content, change in volume, and possible atrophy of the pancreas to determine predictors of diabetes and inform appropriate precautions to consider, optimal pancreatic removal in terms of cost-benefit ratio, and proper management of the condition.

Ethnic Diversity of the pathophysiology of β-Cell Dysfunction

Lidia Szczepaniak, Ruchi Mathur, Edward Szczepaniak, Ildiko Lingvay

Objective: Type 2 diabetes is highly prevalent in Hispanic and Black minorities. Studies suggest that the pathophysiology of type 2 diabetes is different among ethnic groups in regards to the severity of beta-cell dysfunction as well as ectopic fat distribution. We evaluated the ethnicity-specific relationship between pancreatic triglyceride content (pTG) and beta-cell function in non-Hispanic-Black, non-Hispanic-White, and non-Black-Hispanic women.Research Design and Methods: We assessed glucose tolerance (by oral glucose tolerance test), insulin secretion, insulin sensitivity (by frequently sampled intravenous glucose tolerance test), and pTG in vivo (by 1H MR spectroscopy).Results: In non-Black-Hispanics and non-Hispanic-Whites the disposition index (DI) was inversely correlated with pTG: DI=251/pTG+660, R2=0.4088, suggesting beta-cell function impairment with increasing pTG. In non-Hispanic-Blacks DI was directly proportional to pTG: DI=328*pTG+430, R2=0.4472, suggesting beta-cell function enhancement with a narrow-range of increased pTG.Conclusions: Pancreatic TGs and beta-cell function are directly associated and this association is ethnicity-specific.

Gastroenterology

Anti-vinculin Antibodies: Multicenter Validation of a Diagnostic Blood test for Irritable Bowel Syndrome

Mark Pimentel, Christopher Chang, Anthony Lembo, Walter Morales, Kathleen Shari Chua, Stacy Weitsman, Emily Marsh, Zachary Marsh

Data have accumulated that a significant portion of irritable bowel syndrome (IBS) cases begin after acute gastroenteritis. Human and animal work suggests that exposure to acute gastroenteritis leads to small intestinal bacterial overgrowth (SIBO) through neuropathic events. Cytolethal distending toxin B (CdtB) from bacteria known to cause gastroenteritis is important in this process through molecular mimicry and autoantibodies to vinculin (cell migration and adherence protein found predominantly on nerves and epithelium). In this multicenter study, we assess anti-vinculin antibodies as a predictor of IBS compared to healthy subjects and inflammatory bowel disease (IBD). Methods: Subjects (18-65 yrs) with Rome positive IBS were recruited from Cedars-Sinai Medical Center and Beth Israel Deaconess Medical Center. Subjects were assessed for symptoms and demographics followed by collection of sera. Subjects were excluded if they had concomitant GI disease, previous GI surgery, adhesions, unstable thyroid disease, diabetes, or HIV. Healthy controls were recruited based on the completion of a GI symptom questionnaire. On this questionnaire, subjects had to have marked <10 for bloating, diarrhea, abdominal pain, and constipation inclusive on a 0-100 VAS. Subjects with inflammatory bowel disease were recruited from an expert tertiary care medical center. Subjects with Crohn's disease or ulcerative colitis were excluded if there was a history of biologic therapy and current prednisone use. Serum from all 3 groups was used to perform and ELISA to determine antibodies to human recombinant vinculin. Results: In total 165 IBS, 30 IBD and 26 healthy control subjects were evaluated. Demographics were similar between groups. Overall, IBS had a significantly greater optical density in the ELISA for anti-vinculin antibodies compared to IBD and healthy subjects. Comparing the two major centers for IBS recruitment, results from both centers were similarly abnormal (P=NS). Interestingly, subjects with a history of acute gastroenteritis, even higher levels of antibodies were seen (P<0.05).

Early Infliximab trough levels predict persistent remission in pediatric IBD Patients

Namita Singh, Casey Rosenthal, Gil Melmed, James Mirocha, Silvia Callejas, Bhavna Tripuraneni, Sharmayne Farrior, Shervin Rabizadeh, Marla Dubinsky

Background: Low infliximab (IFX) trough levels and high anti-infliximab antibodies (ATI) levels are associated with loss of response to IFX. It is unknown whether IFX and ATI levels drawn prior to loss of response predicts long term IFX efficacy. Methods: A prospective cohort of 58 pediatric IBD patients receiving IFX had IFX and ATI levels drawn at weeks 14 and 54 or at early termination. Primary outcome was week 54 persistent remission (PR), defined as being in remission and not undergoing IFX dose or

frequency intensification, prior to our Week 54 endpoint. Univariate and multivariable analyses were used to determine associations between IFX14 and ATI14 levels as well as clinical and laboratory characteristics and week 54 outcomes. Positive and negative predictive values for IFX14 cut-off points were evaluated. Results: Eight patients (13%) were primary non-responders, stopping IFX before week 14. Of the 50 patients entering maintenance at week 14, 4 discontinued IFX prior to week 54. IFX14 trough level (p=0.03), baseline CRP (p=0.021) and week 14 CRP level (p=0.0007) were associated with PR. Multivariable analysis confirmed IFX14 association (p = 0.05) with PR. An IFX14 level of $\geq$5µg/ml had a positive predictive value for week 54 PR of 83.3% (p= 0.016). Conclusions: IFX levels at week 14 predicted week 54 outcomes; with a minimum trough level of 5µg/ml strongly predictive of PR. Assessment of IFX levels prior to the first maintenance dose may be warranted to optimize dosing prior to loss of response to maximize long term benefit of IFX.

Vinculin expression is reduced in an animal model of post-infectious IBS

Stacy Weitsman, Zachary Marsh, Emily Marsh, Walter Morales, Gene Kim, Christopher Chang, Mark Pimentel

Using a recently validated animal model of post-infectious IBS after exposure to Campylobacter jejuni 81-176, we have demonstrated that rats develop altered bowel form and small intestinal bacterial overgrowth similar to human IBS. We have since demonstrated that in humans and rats, IBS is related to the development of autoimmunity to vinculin (an important cell membrane cytoskeletal protein) from molecular mimicry and exposure to C. jejuni cytolethal distending toxin B. In this study we assess vinculin expression in this post-infectious rat model.Methods: Sprague-Dawley rats were divided into 3 groups. Group 1 rats served as controls (n=20). Group 2 rats were gavaged with 108cfu/mL C. jejuni 81-176 as adults (J-/A+). Group 3 rats were gavaged with C. jejuni as a juvenile and then again 2 months later as an adult a second time. For infected rats, they were euthanized 3 months after clearance of C. jejuni. At the time of euthanasia, sections of small bowel (duodenum, jejunum, and ileum) were ligated and contents for total bacterial contents by qPCR as previously described. A segment of mid small bowel was also obtained and retained in RNA later. After homogenizing, extraction of RNA and conversion to cDNA, qPCR was used to determine the level of vinculin in the bowel wall after normalizing for β-actin. The level of vinculin was assessed based on the number of C. jejuni infections and the presence or absence of SIBO in this animal model.Results: Based on normal bacterial levels in the small bowel segments of normal subjects, SIBO was identified in 26% and 46% of rats with single and double exposure to C. jejuni. Overall, vinculin expression was reduced in small bowel of rats exposed to C. jejuni (0.058±0.0053) compared to control rats (0.087±0.0053) (P<0.001). Furthermore, there was a greater reduction of vinculin with two exposures to C. jejuni compared to a single exposure (see figure) (P<0.0001). There was also a trend to lower vinculin expression in rats with SIBO (P=0.05) (figure).Conclusions: Vinculin expression is reduced by exposure to C. jejuni. This reduction is dependent on the number of exposures to C. jejuni with greater reduction in rats that have been exposed to C. jejuni twice. Finally, SIBO is associated with a lower level of vinculin expression. Vinculin may be important in the pathogenesis of post-infectious IBS.

Circulating antibodies to cytolethal distending toxin B correlates with the development of small intestinal bacterial overgrowth in a rat model of post-infectious IBS

Stacy Weitsman, Walter Morales, Gene Kim, Emily Rooks, Christopher Chang, Mark Pimentel

In a recent validated animal model of post-infectious irritable bowel syndrome (IBS), rats develop altered stool form, increased rectal lymphocytes, reduced interstitial cells of Cajal (ICC) and small intestinal bacterial overgrowth (SIBO) three months after acute exposure to Campylobacter jejuni 81-176. Animals infected with a mutant C. jejuni (insertional deletion of the cytolethal distending toxin B (CdtB) gene) had a mitigated phenotype. In another study, rabbit anti-CdtB was found to exhibit affinity for enteric ganglia and ICCs, suggesting molecular mimicry. In this study, we examine the level of serum anti-CdtB antibodies in our rat model of post-infectious IBS and correlate these with the development of SIBO. Methods: Male Sprague-Dawley rats (n=100) were obtained as infants and randomized to three groups. The first group was gavaged with C. jejuni 81-176 (108cfu/mL) as juveniles and two months later as adults (J+/A+). The second group was gavaged with C. jejuni only as adults (J-/A+). The third group was never exposed to C. jejuni (controls). Three months after the adult infection all rats were euthanized. After euthanasia, segments of ileum, jejunum and duodenum were ligated and removed as previously described (Chatterjee, et al). From each bowel segment, DNA was extracted from luminal contents and qPCR using universal bacterial primers was used to determine the presence or absence of SIBO. SIBO was defined as bacterial counts in excess of 2 standard deviations above mean of controls for each segment. At euthanasia, blood was taken and serum isolated. A 96 well plate was coated with CdtB to which rat serum was added and incubated for 90 minutes. Wells were washed and incubated with a fluorescent secondary antibody and read on a plate reader. Results: ELISA for detection of anti-CdtB in serum of control rats demonstrated an optical density (OD) of 1.27±0.15. All rats exposed to C. jejuni had a greater OD of 1.73±0.12 ($P<0.05$). In the J-/A+ group, the single exposure to C. jejuni resulted in SIBO in 26% of rats. In J+/A+ double exposed rats, SIBO was seen in 46% ($P<0.05$). Anti-CdtB was greater if rats had SIBO irrespective of whether they had a single (1.79±0.31) or double exposure (2.02±0.22) to C. jejuni. Rats that did not have SIBO had titers <1.7. Plotting the level of bacteria in the ileum against the ELISA findings demonstrated a correlation between levels of bacteria and anti-CdtB ($R=0.3$, $P<0.05$).Conclusions: Antibodies to CdtB develop after exposure to C. jejuni but appear to develop in a pattern that relates to the development of SIBO more than the number of exposures to C. jejuni. Based on the affinity for ICC and ganglia, we speculate that these antibodies are important to the pathophysiology of IBS perhaps by affecting gut motor function leading to SIBO.

Gene Therapy

Developing Stem Cell-based anti-Hepatitis C Therapy for Liver Regeneration

Joseph Ignatius Irudayam, Seigo Hatada, Songyang Ren, Weidong Xiong, Vaithi Arumugaswami

Decompensated liver disorders caused by viral hepatitis and alcohol abuse require liver transplantation. Hepatitis C virus (HCV) infection is the leading reason for liver transplantation. Our overall goal is to develop a patient-specific stem cell based anti-HCV therapy to enable regeneration of damaged livers. HCV infection can be inhibited by targeting entry, genome replication and egress steps of viral lifecycle. HCV utilizes CD81, a cell surface tetraspanin protein, for entering into cell. Silencing CD81 expression through RNA-interference (RNAi) resulted in inhibition of HCV replication. We focus on generating CD81 knockout induced pluripotent stem cell (iPSC) lines which can confer resistance to HCV infection. We utilize Cas 9 system for specifically deleting CD81 gene from chromosome in 19th chromosomes of iPSC line. We constructed three guided RNA (gRNA) which can specifically target Exon 5 of CD81. We have generated donor vector with homologous sequences of CD81 gene and puromycin selection marker. As a proof of concept, we tested the targeting efficiency of cas 9 system using Huh-7.5.1 cells that supports HCV infection. We have observed a CD81 knockout (KO) efficiency of 10-25% in Huh-7.5.1 cells by flow cytometry. Subsequently, we tested the CD81 Cas9 system in a iPSC line. The iPS cells were transfected with hCas9, gRNA and donor vector plasmids by electroporation method. After four days, we sorted out CD81 double negative population (1.8 to 3.0%) by flow cytometry. We got 42 individual iPSC clones from the sorting experiments. We verified all the clones CD81 receptor expression by flow cytometry analysis. Total six clones showed possible reduction of CD81 KO population 53 % to 56 % and 61 to 69 % respectively. Further, these clones need to be verified by PCR screening and blotting techniques. These results demonstrate that the cas 9 system efficiently targets CD81 gene in iPSC cell line.

Genetics

Gene expression profile in ovarian stroma in BRCA1 carriers and age-matched controls

Natalia Babkina

Background: Hereditary breast and ovarian cancer caused by germline mutations in cancer susceptibility genes BRCA1 and BRCA2 is the most common, highly penetrant cancer syndrome. Mutations in BRCA1 confer higher risk of ovarian cancer and earlier age of onset. BRCA1 participates in transcription regulation and its haploinsufficiency may lead to alterations in gene expression profile in BRCA1 carriers prior to genomic and histological signs of neoplasia. Initiation of high-grade serous carcinoma occurs in the ovary, fallopian tube, or peritoneum, but tumor growth and progression is preferential to the ovary. Ovarian stromal fibroblasts may play an important role in initiation and promotion of serous carcinogenesis.Objective: to determine the gene expression profile in ovarian stroma in BRCA1 carriers and age-matched controls and to identify alterations in pathways related to carcinogenesis in BRCA1 carriers.Materials and Methods: Registry of the Gilda Radner Hereditary Cancer Program will be reviewed. 12 premenopausal BRCA1 carriers, age 35 - 50 years old, who underwent risk-reducing salpingo-oophorectomy and had histopathologically normal fallopian tubes and ovaries and 12 age-matched controls who underwent salpingo-oophorectomy for non-oncological indications will be included. Women with personal or family history in the first-degree relative of breast or ovarian cancer will be excluded. Ovarian stroma will be dissected from FFPE samples. RNA will be isolated and sequenced. Sequencing reads will be aligned. The Benjamini-Hochberg step-up method will be applied to control FDR. The list of differentially expressed genes will be generated based on statistical (FDR<0.2) and biological significance (>2-fold change).Anticipated results: The study will provide the proof of principle for identification of gene expression profile in ovarian stroma in BRCA1 carriers and age-matched controls. In BRCA1 carriers alterations in expression profile of the genes that participate in DNA repair and pro-inflammatory signaling are expected, specifically, changes associated with impaired DNA double-strand breaks repair and activation of cell senescence program.

Expression studies of keratoconus corneal buttons reveal abnormalities in the regulation of extracellular matrix and adhesion molecules

Yelena Bykhovskaya, Helen Makarenkova, Yaron Rabinowitz

Keratoconus (KC)is a non-inflammatory corneal disorder of complex genetic inheritance characterizedby progressive corneal thinning. Genes encoding proteins of extracellularmatrix (ECM), including collagens, and adhesion molecules have been proposed tocontribute to normal variation of central corneal thickness and a number of corneal diseases, including KC. We used Human Extracellular Matrix & Adhesion Molecules RT ProfilerPCR Array to identify changes in the expressionbetween corneal tissue obtained from KC patients, patients with cornealopacities, and normal controls using total RNA was

extracted from cornealbuttons obtained during keratoplasty. Unsupervised clustering analysis revealedseveral distinct expression signatures between KC patients, patients withcorneal opacities, and normal controls. Interestingly comparison of KC patientsand patients with corneal opacities showed a substantial difference in geneexpression, suggesting KC specific alterations in ECM composition. Comparisonof keratoconus and control corneas with thresholds of fold change of 1.5 orgreater and p-value of 0.05 or lower, revealed twenty-five differentiallyexpressed genes. Notably, twofold more genes (seventeen) were found to besignificantly downregulated than to be upregulated (eight). Among transcripts downregulatedin KC patients we identified several collagens (COL5A1, COL6A1, COL7A1, COL11A1),integrins (ITGA1, ITGA5, ITGAM, INTA8), metalloproteinases (ADAMTS1, MMP2,MMP9), as well as tissue inhibitors of metalloproteinases (TIMP1, TIMP2). Wehave also confirmed significant downregulation of thrombospondin 1 (THBS1) previouslyidentified in the comparative transcriptome analysis of KC corneas. Inaddition, we observed significantly lower expression of fibronectin (FN1), aubiquitous ECM glycoprotein important for tissue repair. Among upregulatedgenes, we identified transforming growth factor beta-induced gene TGFBI(p=0.0009), which plays a role in cell-collagen interactions, and was previouslyidentified in the KC library constructed by our group. TGFBI mutations havebeen linked to several corneal dystrophies and its overexpression in thetransgenic mice results in the abnormal corneas. Based on the results of ourstudy we conclude that a decrease in the expression of multiple collagens andrelated proteins (TGFBI) may be largely responsible for the thinning of thecollagenous corneal stroma whereas decrease in FN1 and THBS1 may potentially leadto inflammation and affect corneal repair. These expression results provide furthersupport to the potential deregulation of ECM and adhesion proteinsas a pathogenic factor in the development of KC.

Impact of Genetic Ancestry on the Incidence of Sudden Cardiac Death: The Oregon Sudden Unexpected Death Study

Adriana Huertas-Vazquez, Kyndaron Reinier, Carmen Teodorescu, Audrey Uy-Evanado, Jo Ayala, Katherine Jerger, Harpriya Chugh, Karen Gunson, Jonathan Jui, Sumeet Chugh

Although sudden cardiac death (SCD) is a major public health problem, the incidence of SCD in non-white populations has not been sufficiently investigated. We estimated the incidence of SCD from the established Oregon Sudden Unexpected Death Study (Oregon SUDS) using ancestry informative markers (AIMs).Age-specific and age-adjusted incidence rates were calculated for 1,149 cases of SCD from Multnomah County, Oregon (2002-2005). In a subset of 966 cases, we also estimated ancestry using AIMs, employing maximum likelihood estimates.Based on reported ethnicity, age-adjusted rates were highest among blacks (123 per 100,000) and lowest among Asians (36 per 100,000), and were 59 per 100,000 for non-Hispanic whites and 52 per 100,000 for Hispanics. Blacks were younger than whites at the time of SCD (p=0.007) and had a higher prevalence of diabetes (p=0.0001). Based on the AIMs findings we observed that 2.5% of individuals of reported non-Hispanic white ethnicity were likely of Hispanic origin. After correcting for AIMs, SCD incidence was significantly higher among Hispanics (110 per 100,000).These findings indicate that 1) misclassification of reported ethnicity may result in

underestimation of SCD rates among Hispanics and 2) blacks had more than double the incidence of SCD compared to whites.

Imaging

Fractal dimensions and lacunarity of nuclear distribution separate benign tissue and cancer grades in radical prostatectomies

Arkadiusz Gertych, Caleb Cheng, Zhaoxuan Ma, Sanica Bhele, Sambit Mohanty, Yung-Tien Chu, Daniel Luthringer, Mahul Amin, Beatrice Knudsen, James Mirocha

Background: Lacunarity (L) and fractal dimensions (FD) are quantitative measures to assess the complexity of an image. They describe repetitive patterns and structural heterogeneity of objects filling the image space. Since the arrangement of nuclei differs between benign and malignant glands, we hypothesize that FD and L can capture topology of nuclei and thus distinguish benign glands, Gleason grade 3 (G3), G4 and cancer. Design: Images were collected from 12 cases of radical prostatectomy and areas containing primarily benign epithelium (BE, n=25), cancer G3 (n=37), G4 (n=29) or stroma (ST, n=24) were identified by two pathologists. A binary nuclear map and a hematoxylin image were determined for each area. FracLac plugin installed on ImageJ platform was used to calculate FD and L in nuclear masks (FD(n), L(n)) and in hematoxylin images (FD(h), L(h)) in BE, G3 and G4 areas. ST served as a reference. Results were evaluated by the Tukey's Studentized Range test. Results: Measurements of FD(n) and L(h) were statistically different between areas of G3 and G4, G3 and BE and G4 and BE, but did not reach statistical significance in FD(h) and L(n). All four measures were significantly different comparing cancer and BE to ST.

Immunology/Infection/Inflammation

B-lymphocyte induced maturation protein 1 (Blimp-1) is required to limit the number of IL17A-producing CD4+ T cells in vivo

Rashmi Bankoti, Soofia Salehi, Luciana Benevides, Michael Couse, Joao Silva, Deepti Dhall, Eric Meffre, Stephan Targan, Gislaine Martins

Blimp-1 is a transcription factor required for embryonic development and immunity. Mice with T-cell-specific deletion of Blimp-1 (Blimp-1CKO) develop severe intestinal inflammation apparently mediated by CD4+Th cells, but little is known about Blimp-1's role in regulating Th cell differentiation in vivo. Here we show that Blimp-1 restrains Th cell production of IL17 and IFNγ in vivo. Blimp-1CKO mice accumulate IL17 and IFNγ-producing TCRβ+CD4+cells in lymphoid organs and intestinal mucosa. Increased numbers of IL17-producing Th cells are also observed in WT and Blimp-1CKO-mixed BM chimeric mice containing WT regulatory T (treg) cells, suggesting a Treg-independent intrinsic role for Blimp-1 in constraining IL17production. Blimp-1-/-naive CD4+Tcells are more prone to differentiate into IL17+IFNγ+ cells and cause severe colitis when transferred to Rag1-/- mice. In vitro-stimulated Blimp-1-/- CD4+T cells show increased expression of il17a and other Th17-related genes, suggesting that Blimp-1 could inhibit transcription of Th17 genes. Using ChIP assays we identified at least one site at the il17a gene that can be bound by Blimp-1 in Th cells, suggesting that Blimp-1 could function as a direct repressor of il17a. Single cell analysis reveals that Blimp-1 expression is selectively regulated during Th differentiation and directly correlates with suppression of Th17 traits in Th1cells. Collectively, these results establish a new role for Blimp-1 in regulating IL17 production in vivo.

MyD88 signaling in both DCs and endothelial cells are required for high-fat diet-induced atherosclerosis

Shuang Chen, Kenichi Shimada, Timothy Crother, Wenxuan Zhang, Ganghua Huang, Moshe Arditi

Background: Studies from our laboratory and others indicate that Innate Immune Receptors TLR2, TLR4, and the adaptor molecule MyD88 promote development of atherosclerosis in hypercholesterolemic mouse. Objective: To investigate the requirement of MyD88 signaling in hematopoietic in acceleration of atherosclerosis. Methods and Results: In the 4 groups of Bone Marrow chimeric mice, quantification of the lesion area of aortic sinus plaques and lipid content revealed a significant reduction in aortic root lesion size and lipid content in irradiated ApoE-/- mice reconstituted with ApoE-/-/MyD88-/- BM compared to reconstitution with ApoE-/-MyD88+/+ BM despite similar cholesterol. Furthermore, ApoE-/-/MyD88-/- recipient mice reconstituted with ApoE-/-/MyD88+/+ BM also demonstrated a significant reduction in lesion size, and lipid content in aortic sinus, compared with ApoE-/-/MyD88+/+ BM recipients. Lack of MyD88 in either stromal or hematopoietic cells resulted also with a significant decrease in serum IL-12p40 and IL-6. In addition, we generated mice with the specific loss of MyD88

gene only in EC using the cre flox system,Myd88flox/flox Tie2Cre mice and bred these mice onto the ApoE-/- background. The ApoE-/-/Tie2Cre mice had significantly reduced lesion size in aortic sinus and Aorta en face preparations. We crossed the CD11c transgenic MyD88 mice with ApoE-/- mice to specifically investigate the role of MyD88 signaling in CD11c+ DCs. While ApoE-/-;MyD88-/- double KO mice had very little lesion development, the ApoE-/-;Myd88-/-CD11cTg mice had significantly increased lesion in the aortic sinus and aorta. Conclusion: MyD88 signaling in both hematopoietic cells, especially DCs, as well as stromal cells, such as ECs, play an important role in diet-induced acceleration of atherosclerosis.

Divergent effects of Autophagic Components ATG5 and ATG16L1 on Chlamydia pneumoniae Infection and Replication

Timothy Crother, Kenichi Shimada, Shuang Chen, Moshe Arditi

Chlamydia pneumoniae (CP), an obligate intracellular pathogen, is responsible for up to 20% of community-acquired pneumonia (CAP). CP infection is associated with the onset and or exacerbation of many chronic inflammatory diseases, including atherosclerosis, asthma, COPD, and Alzheimer's Disease. Autophagy is the process by which cellular components gets recycled. Autophagy has been shown to be involved in both bacterial killing and adaptive immunity. However, the role of autophagy in CP infection is currently unknown. We have found that ATG5, a component of the autophagic machinery, is required for proper CP replication in vitro. ATG5 deficient MEFS have smaller and reduced numbers of Chlamydial inclusions. However, ATG16L1, another autophagy protein that is part of the same complex as ATG5, has an inhibitory effect on CP growth. ATG16-/- macrophages, and ATG16+/- MEFS have increased CP numbers and larger inclusions. These divergent data suggest that the effects of ATG5 and ATG16L1 are not due to traditional autophagic mechanisms. Our preliminary data indicates that ATG5 is required for nutritional requirements during CP infection and that excess energy and amino acid supplements can reverse the effects of ATG5 deficiency. However, the mechanism of ATG16L1's action on CP growth remains unclear and requires further studies.

IL-1α released from Necrotic Alveolar Macrophages mediates LPS induced Acute Lung Injury via MyD88 signaling on Endothelial Cells

Jargalsaikhan Dagvadorj, Kenichi Shimada, Timothy Crother, Shuang Chen, Heather Jones, Gantsetseg Tumurkhuu, Wenxuan Zhang, Moshe Arditi

We investigated the role of IL-1 and endothelial cell (EC) MyD88 signaling in LPS-induced acute lung injury (ALI). IntratrachealLPS induced IL-1α -dependent neutrophil recruitment and vascular leakage, and these effects were due to the release of proIL-1α from necrotic alveolar macrophages (AM). LPS induced AM necrosis by depleting intracellular ATP in a P2X7 receptor-dependent manner. We generated EC specific MyD88 deficient mice (ECMyD88-/-) to examine downstream signaling of IL-1a.

Neutrophil recruitment and pulmonary vascular leakagewere significantly decreased after LPS instillation in ECMyD88-/- mice compared to controls. The VE-cadherin expression was reduced in EC after LPS instillation in control mice but not in ECMyD88-/- mice. Additionally, ECMyD88-/- infected with Klebsiella pneumonia had reduced neutrophil recruitment with increased bacterial burden and mortality. These results demonstrate a key role for proIL-1α, released after AMnecrosis, in inducing disruption of endothelial cell junctions throughEC-MyD88 signaling and neutrophil migration during LPS-induced ALI.

High Levels of Plasma IL-21 Together with EBV-specific Cytotoxic T Cells (EBVTc) May Contribute to Avoidance of Post-transplant (Tx) Lymphoproliferative Disorder (PTLD) in Pediatric Kidney Tx Patients (pts) with Persistent EBV Infection (PEBV)

Shili Ge, Dechu Puliyanda, Artur Karasyov, Junji Watanabe, Elaine Kamil, Darly Lovato, Stanley Jordan, Mieko Toyoda

Background: PEBV infection is associated with high risk for development of PTLD. Recent studies report a critical role for IL-21 in sustaining viral-specific Tc activity during chronic viral infections. Here we measure the plasma levels of anti-viral cytokines (IL-21, IFNg, IL-17A) (pg/ml) and EBVTc in pediatric kidney Tx pts and correlated these findings with EBV infection and PTLD status. Method: Archived plasma obtained at multiple time points post-Tx from 48 pts who had PEBV (10), acute EBV (6, AEBV) and no EBV viremia (32, NEBV) were submitted for cytokine luminex assay. EBV DNA was quantified by PCR, and EBVTc by an intracellular cytokine flow cytometry. PEBV was defined as EBV viremia >10 copies/PCR for >3months. Results: Mean (155±218 vs. 12±8, $p<0.05$) and peak (201±260 vs. 20±10, $p<0.05$) EBV DNA levels were significantly higher in PEBV than AEBV groups. The IL-21 (199±108 vs. 94±51, $p<0.05$) and IL-17A (76±41 vs. 43±59, $p<0.05$) levels were significantly higher in PEBV than NEBV. IFNg showed a similar trend (104±133 vs. 67±251, $p=0.10$), but not statistically significant. The IL-21 levels in AEBV were 119±54 and were also significantly higher than those in NEBV pts ($p<0.05$), but were significantly lower than those in PEBV pts ($p<0.05$). There was no difference in the IL-17A and IFNg levels between AEBV and NEBV. The IL-21 levels significantly increased with the EBV DNA levels (0 copy: 101±67, 0-<5: 139±94, 5-50: 170±114, >50: 180±97: $p<0.05$), but IL-17A or IFNg did not. Among 9 PEBV pts w/ EBVTc results, all showed EBVTc (+) and anti-EBV antibody (+). None of AEBV and PEBV developed PTLD. Conclusions: 1) EBV DNA levels were significantly higher during PEBV than AEBV, 2) IL-21 levels increased during PEBV and was much higher than seen with AEBV, 3) The levels of IL-21, but not IL-17A or IFNg, were significantly higher at high EBV DNA levels observed during PEBV. These results demonstrate an important role of IL-21 during PEBV and suggest that EBVTc together with IL-21 and anti-EBV antibody control EBV proliferation during PEBV and likely preventing PTLD.

The NLRP3 inflammasome is required for the development of hypoxemia in an LPS/mechanical ventilation model of acute lung injury

Heather Jones, Timothy Crother, Mahdulika Jupeli, Shuang Chen, Jargalsaikhan Dagvadorj, Moshe Arditi, Kenichi Shimada

Rationale: Interleukin 1b is a potent pro-inflammatory cytokine often implicated in the pathogenesis of acute lung injury (ALI). We hypothesized that the combination of lipopolysaccharide (LPS) and mechanical ventilation (MV) leads to IL-1b secretion and the development of ALI, and that this process is dependent on activation of the NLRP3 inflammasome. Methods: We used a two-hit murine model of ALI in which both inhaled LPS and MV were required for the development of hypoxemia, pulmonary neutrophil infiltration, and alveolar leakage. Measurements and Main Results: NLRP3-deficent and Caspase-1-deficient mice had significantly diminished IL-1b levels in bronchoalveolar lavage fluid and were specifically protected from hypoxemia, despite similar alveolar neutrophil infiltration and leakage. The IL-1 receptor antagonist Anakinra blocked the development of hypoxemia without affecting neutrophil infiltration or alveolar leakage. MV increased alveolar macrophage apoptosis, a trigger of NLRP3 inflammasome activation. Conclusions: NLRP3 inflammasome activation and IL-1β production play a key role in the development of hypoxemia in this two-hit model of ALI. Blocking IL-1 signaling in this model ameliorates hypoxemia without affecting neutrophil infiltration and alveolar leakage, disassociating these readouts of ALI. MV causes alveolar macrophage apoptosis, a key step in activation of the NLRP3 inflammasome and production of IL-1b.

IBD-associated PRDM1 (PRDI-BF1/BLIMP-1) polymorphisms and CD4+ T cell function in the human intestinal mucosa

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Inflammatory bowel disease (IBD) is a debilitating condition characterized by a spectrum of clinical manifestation, which etiology still remains unknown. IBD pathogenesis is mediated by an abnormal immune response to microorganisms present in the intestinal microbe flora, which leads to a chronic inflammatory response. CD4+ T helper effector (Teff) and regulatory (Treg) lymphocytes play important role in IBD pathogenesis, but the mechanism underlying their effector function in IBD are not fully understood. B lymphocyte-induced maturation protein-1 (PRDI-BF1/BLIMP-1) is a transcriptional regulator previously shown to play a crucial role in regulating the function of Teff and Treg cells. Our previous studies in mice demonstrated that conditional deletion of BLIMP-1 in T cells leads to the spontaneous development of IBD [Martins et al, Nat Immunol. 2006], associated with dysregulated cytokine production [Martins et al, J Exp Med. 2008], including increased production of inflammatory cytokines such as IL17A, IL17F and IFNγ [Salehi et al, J.Immunol. 2012]. In addition, recent Genome Wide Associated Studies (GWAS) indicate strong association between the presence of specific single nucleotide polymorphisms (SNPs) in the gene encoding PRDM1 and several chronic inflammatory conditions, including IBD (Fig. 1). In order to begin to evaluate whether or not BLIMP-1 could be involved in controlling the function of T cells, we have evaluated BLIMP-1 expression in different subsets of CD4+ T cells from peripheral blood from control individuals. This analysis revealed that BLIMP-1 expression in

these cells mimics the pattern previously described in murine cells (Fig. 2A). In addition, we also found that BLIMP-1 protein is highly expressed in CD4+ T cells from the intestinal mucosa from healthy individuals (Fig. 2B). In the present study we propose to utilize a combination of cellular and molecular assays (see study design below) to determine whether or not BLIMP-1 can control the function of mucosal T cells and evaluate the functional implication of PRDM1 SNPS that could underlie the association of this gene with IBD. We will work with primary T cells obtained from peripheral blood and intestinal mucosa from control subjects and IBD patients (Tables I-II) from an extensive database previously established at our institution.

Development of Lung ILC2 Cells Requires TOX

Corey Seehus, Parinaz Aliahmad, Brian de la Torre, Jonathan Kaye

Recently, a subset of innate lymphoid cells that produce type 2 cytokines (ILC2) has been identified. These cells, present in both human and murine lung, are not only important for immunity to parasites, but also play a protective role in maintaining barrier function during influenza infection and a pathologic role in airway hyperresponsiveness. TOX is a member of the HMG-box family of DNA-binding nuclear proteins and is necessary for the proper development of multiple lineages of cells in the immune system, including ILC1 and ILC3 subsets of innate lymphoid cells (NK and lymphoid tissue inducer cells, respectively). Here we have asked whether TOX is also involved in development of ILC2 cells. Using a reporter strain of knock-in mice that expresses tdTomato fluorescent protein under the regulatory control elements of the Tox locus, we find that ILC2 cells in the lung express TOX. Moreover, there is a severe depletion of ILC2 cells in the lungs of TOX-deficient mice. ILC2s have recently been shown to develop from common lymphoid progenitors (CLPs) in the bone marrow through a lineage restricted precursor stage (ILC2p). Although TOX-deficient mice possess CLPs in normal numbers, ILC2p cells are absent in TOX-deficient animals suggesting that development of ILC2 cells is blocked at a very early stage in development. In vitro experiments with CLPs isolated from TOX-deficient animals indicate that this developmental block is cell intrinsic. Experiments are in progress to assess the mechanism in which TOX-deficiency leads to the absence of ILC2 cells in the murine lung.

Novel role of Rip2 in T cells: Rip2 negatively regulates Th17-dependent chronic lung and vascular inflammation

Kenichi Shimada

While Rip2 plays a role for intracellular pathogen sensing, its role in T cells is unknown. We found that Rip2 deficiency results in enhanced Th17 skewing in both in vitro and in vivo models. This skewing is intrinsic to Rip2-/- T-cells as naive CD4 Rip2-/- T-cells transferred into Rag1-/- mice preferentially differentiated into Th17 while WT T-cells did not. Expression Array data revealed that Rip2-/- T-cells expressed greater amounts of RORalpha compared with WT T-cells during pathogenic Th17

differentiation, but not under classical Th17 differentiation conditions. To investigate the functional impact of this observation, we used two different experimental models. Rip2-/- mice had a severe chronic lung inflammation following Chlamydia pneumoniae infection, which was not observed in Il17a-/-/Rip2-/- mice. Additionally, when irradiated Ldlr-/- mice received Rip2-/- bone marrow, we found a significant increase in atherosclerosis, which was not observed using Il17a-/-/Rip2-/- BM. Our study demonstrates a novel, intrinsic role for Rip2 in T-cells whereby Rip2 negatively regulates Th17 development.

Plasma Soluble IL-6 Receptor (SIL-6R) levels are significantly elevated during antibody-mediated rejection (ABMR) in highly HLA-sensitized renal allograft recipients (HS PTS)

Bongha Shin, Junji Watanabe, Shili Ge, Ashley Vo, Stanley Jordan, Mieko Toyoda

IL-6/sIL-6R interactions have pleiotropic effects on immunity and inflammation. We measured plasma sIL-6R in renal transplantation (Tx) HS patients with ABMR, cellular-mediated rejection (CMR), acute tubular necrosis or calcineurin inhibitor toxicity. Archived samples obtained from 46 patients who received desensitization followed by a kidney transplant and sera from 84 normal individuals were submitted for sIL-6R ELISA. Pre-Tx average sIL-6R levels in these patients were similar to those in normal individuals. However, ESRD patients showed wide range of sIL-6R levels. Post-Tx sIL-6R levels at ABMR or CMR were similar to those in normal individuals, and there was no significant difference at ABMR vs. CMR. However, there is a trend that sIL-6R levels increased at bacterial, viral infection and ABMR when the levels were compared within a patient. This shows: 1) The wide range of pre-Tx sIL-6R levels observed in ESRD patients awaiting Tx may represent inflammatory condition in patients, 2) Elevated levels of plasma sIL-6R observed at bacterial or viral infection may represent a risk for inflammation mediated allograft injuries in transplant recipients, and 3) Elevated sIL-6R levels without any evidence of bacterial and viral infection may represent a possible ABMR as it was observed in some of patients with ABMR.

TL1A signaling enhances the induction of IL-9 producing T cells via STAT6 and BATF signaling pathways

Masato Tsuda, Lisa Thomas, Brenda Salumbides, Michelle Wong, Jordan Nunnelee, Marie Bowsman, Stephan Targan, Kathrin Michelsen

TL1A, a member of the TNF superfamily, augments effector T helper (TH) 1, TH17, and TH2 responses in several chronic inflammatory conditions such as chronic colitis, experimental autoimmune encephalomyelitis, and allergic lung inflammation. However, it remains to be elucidated if TL1A has an impact on the development and function of other TH subsets. Here, we show that TL1A enhances the differentiation of TH9 cells, a recently identified TH subset that produces IL-9 and promotes allergic lung inflammation. TL1A together with TGF-b1 and IL-4 induced significantly higher level of IL-9 expression early on and sustained IL-9 production. In addition, TL1A enhanced the expression of other TH9-related

cytokines such as IL-10, IL-21, and IL-13. During TH9 differentiation, TL1A signaling led to STAT6 phosphorylation and upregulated the expression of the transcription factors BATF, GATA3, and IRF4 which are important for TH9 differentiation. Furthermore, Stat6-/- T cells stimulated with TL1A under TH9 condition did not upregulate BATF expression and produced significantly lower level of IL-9, IL-10, and IL-13 compared to WT T cells. Taken together, our data show that TL1A promotes the differentiation of TH9 cells through STAT6 and BATF signaling pathways, indicating a novel role for TL1A in developing pathogenic TH9 effector cells.

NLRP3 inflammasome activation plays an important role in the Chlamydia pneumoniae induced acceleration of foam cell formation

Gantsetseg Tumurkhuu, Timothy Crother, Kenichi Shimada, Moshe Arditi, Shuang Chen

Background and Objective C. pneumoniae (Cp) promotes FCF in the presence of oxLDL, here we investigated the role of the Nlrp3 inflammasome in this process.Methods Peritoneal macrophages from wild-type (WT), and Nlrp3-/- mice were treated with live Cp (MOI=5:1) and/or ox-LDL (25 μg/ml). Also, the cells were treated with IL-1R antagonist, Anakinra (10 μg/ml) or LXR agonist, GW3968 (1 μM). Intracellular lipids was assessed by Oil Red O staining. The expression of selected genes (IL-1α, IL-1β, TNF-α, LXR-α, ABCA1, ABCG1 and CD36) were examined. Transcriptional host cell responses to Cp infection and/or oxLDL were analyzed by microarray.Results Co treatment with Cp and oxLDL for 24h in WT macrophages resulted FCF, which was attenuated in Nlrp3 KO and in IL-1R antagonist treated WT cells. The expression of LXR-α, ABCA1, and ABCG1 were higher in Nlrp3-/- macrophages compared to WT cells. But both CD36 and SR-B were up-regulatedbothWT and Nlrp3 KO. mRNA profiling by microarray showed several genes known to activate LXR-α were upregulated and LXR-α inhibitors were downregulated in Nlrp3 KO to the WT macrophages.Conclusion Cp infection facilitatesFCF in the presence of oxLDL by producing IL-1 cytokines, dependent on the activation of the NLRP3 inflammasome.

Abdominal Aorta Dilatation and Aneurysm in Kawasaki Disease Vasculitis Mouse Model. Role of IL-1beta signaling

Daiko Wakita, Yosuke Kurashima, Youngho Lee, Kenichi Shimada, Shuang Chen, Timothy Crother, Thomas Lehman, Michael Fishbein, Moshe Arditi

Background - Kawasaki disease (KD) is the most common cause of acute systemic vasculitis and acquired cardiac disease among US children and causes coronary artery aneurysms (CAA) in 15-25% of untreated patients. Aneurysms due to KD have been reported relatively rarely in other systemic arteries including the abdominal, renal and iliac arteries In a Lactobacillus casei cell wall extract (LCWE)-induced mouse model of KD we have shown that coronary arteritis and vasculitis is an IL-1-driven process. Methods and Results. We measured the diameter of abdominal aorta at 2, 5 weeks following LCWE injection in this

KD model. Over 80% of the mice developed abdominal aorta dilatation at 2 wks with progressively higher dilatation at 5 wks. Some mice showed fusiform and saccular abdominal aneurysms, and dilatations of iliac and renal aorta. Histopathology showed significant intimal proliferation, and myofibroblastic proliferation that infiltrates and destructs the medial elastic laminae, massive inflammatory cell infiltration into media and adventitia (large number of neutrophils and DCs, and small population of CD4+, CD8+ T cells and macrophages). IL-1R KO as well as IL-1beta KO-mice were completely protected not only from coronary arteritis but also from abdominal aorta dilatation/aneurysm. Conclusions - LCWE-induced KD mouse model develops abdominal aorta dilatation (80% incidence) of aneurysms that is also IL-1-dependent. Blocking IL-1 signaling molecules maybe a promising therapeutic target for KD coronary arteritis and systemic arterial injury. Abdominal aorta dilatation and aneurysms in children with KD maybe more prevalent than what has been appreciated until now.

The effects of IFNg and IL17a on Tl1a induced murine gut inflammation and fibrosis

Kori Wallace, Yoshitake Kanazawa, Hong Zhang, Ryan Ichikawa, Jeremy Chen, Stephan Targan, David Shih

TL1a expression is elevated in gut mucosa of some inflammatory bowel disease (IBD) patients and confers a worse prognosis. Mice with constitutive lymphoid TL1a expression (L-TG) demonstrate increased IFNg and decreased IL17a levels in the gut with resulting inflammation, fibrosis, and worsened colitis. To determine the contribution of specific immunologic pathways on TL1a induced murine colitis, L-TG mice were crossed with mice deficient in IFNg (L-TG;IFNgKO) and IL17a (L-TG;IL17KO). The adoptive transfer model of colitis was utilized by transferring naive T-cells from the L-TG and cytokine deficient mice into RagKO recipients. Markers of gut inflammation and fibrosis were measured. Compared to L-TG mice, L-TG;IFNgKO mice had worsened gross pathology and histology, increased immune cell infiltrate, markers of inflammation (CD69, CD44, IL17a, and IL9), and markers of fibrosis (collagen deposition and numbers of activated fibroblasts), with decreased levels of IL10, an anti-inflammatory cytokine. Compared to L-TG mice, L-TG;IL17aKO mice had improved gross pathology and histology, decreased immune cell infiltrate, markers of inflammation, and markers of fibrosis, with increased levels of IL10. These data taken together suggest that TL1a overexpression modulates gut inflammation and fibrosis and that IFNg may negatively regulate TL1a production, while IL17a positively regulates TL1a production.

Inflammatory cytokines were elevated during kidney graft injuries in HLA-sensitized (HS) renal transplant patients (TX PTS)

Junji Watanabe, Ashley Vo, Rafael Villicana, Stanley Jordan, Mieko Toyoda

Background: End-stage renal diseases (ESRD) complicated with HLA sensitization limits graft accessibility and increases the risk of graft injuries, morbidity and mortality after kidney Tx. The precise predictions

and prevention of kidney injuries in this population are still poor because of complex pathogenetic mechanisms. We hypothesize that inflammatory process should accompany with any graft injuries. Here we measure the levels of inflammatory cytokines (IFNg, IL-6, IL-10 and IL-17) in renal Tx pts who had various graft injuries including antibody-mediated (ABMR), cellular-mediated (CMR) rejections, acute tubular necrosis (ATN) and calcineurin inhibitor cytotoxicity (CNI), to determine if those levels can be used as biomarkers for kidney graft injuries.Methods: Archived plasma samples obtained at biopsy (up to 6 months post-Tx) (Bx: 20 AMR, 12 CMR, 5 AMR/CMR, 16 ATN/CNI/others) and post-Bx (up to 2 months post-Bx) time points per pts in 35 kidney Tx pts were submitted for multiplex Luminex assays for IFNg, IL-6, IL-10 and IL-17A. Post-Tx plasma samples from stable pts without Bx were also analyzed as controls. Cytokine concentrations were in pg/mL. Results: IFNg (7.5±2.7 vs 0.5±0.2, p=0.02), IL-6 (33.6±18.7 vs 0.9±0.4, p=0.003), IL-10 (8.3±2.2 vs 1.1±0.5, p=0.02) and IL-17A (6.8±1.5 vs 0.2±0.0, p=0.003) levels were significantly higher at episode compared with those post-Tx in stable pts. No significant difference was observed between types of graft injuries. Furthermore, these elevated levels significantly decreased within 2 months post-episode (IFNg: 52% reduction from the Bx time point, p=0.02; IL-6: 73%, p=0.002; IL-10: 70%, p=0.02; IL-17A: 42%, p=0.007). Conclusions: 1) The increased levels of IFNg, IL-6, IL-10 and IL-17A during kidney graft injuries confirmed involvement of inflammatory process in any types of graft injuries, and 2) these inflammatory cytokines could be potential biomarkers for allograft injuries in renal Tx pts.

Therapeutic Anti-IL6 Receptor Antibody Is Effective in Attenuating Recall Alloantibody Responses in a Mouse Model of Allo-sensitization

Gordon Wu, Irene Kim, Ning-ning Chai, Andrew Klein, Stanley Jordan

Background Alloantibody-mediated rejection (AMR) in pre-sensitized kidney transplant patients is difficult to control due to lack of effective treatment options. We recently found in a mouse model that IL-6R blockage by a mousenized rat-anti-mouse IL-6R mAb (mMR16-1) is effective in suppressing de novo donor-specific antibody (DSA) responses. This study further evaluated the effectiveness of mMR16-1 in recall antibody responses. Method C57BL/6 mice were pre-sensitized with skin allograft (SG) from a HLA.A2 transgenic mouse. At Day 70 the pre-sensitized mice were re-stimulated with a second HLA.A2+ skin graft and divided into 4 different treatment groups, including (1) mMR16-1, (2) control antibody, (3) anti-CD20 and (4) IVIG. Recall alloantibody responses were monitored weekly for 4 weeks by measurement of serum anti-HLA.A2 antibodies. T, B and plasma cells were analyzed in FACS and ELISpot assay. Results All the pre-sensitized mice had moderate levels (113.9-143.9 MFI) of DSA IgG in sera before 2nd skin grafting. Re-sensitization in the control group (n=6) resulted in a recall response featured by a surge of anti-HLA.A2 IgG at day 14 (633+52 MFI) and a peak at day 28 (827+110 MFI). mMR16-1 significantly reduced DSA IgG levels (336+116 MFI at day 14 and 530+136 MFI at day 28, P=0.003, P=0.15, respectively, as compared to the control). In contrast, anti-CD20 treatment resulted in no significant reduction in DSA IgG levels (472+274 MFI at day 14, P>0.05 as compared to control) amid >90% B220+ cell depletion in the spleens. Human IVIG had no effect on DSA IgG levels in the re-sensitized mice (826+368 MFI, P>0.05). FACS analysis showed that anti-CD20 significantly depleted

B220+ B cells in the spleens and BM, but had no effect on CD138+/CD38+ plasmablasts. On the other hand, mMR16-1 has no depleting effect on splenic T-cells, B-cells and CD11b+ monocyte/macrophages in the spleens. There was a reduction in CD138+ plasma cell population in the BM. Conclusion Antibody therapy targeting the IL-6/IL-6R pathway can modulate recall alloantibody responses. This suggests a newly recognized potential and promising strategy in use of tocilizumab for prevention and treatment of alloantibody mediated rejection in organ transplantation.

Evidence that Interleukin-6 Receptor Antibody Suppresses Long-lived Plasma Cells in Alloantibody Recall Response

Gordon Wu, Iren Kim, Ning-ning Chai, Stanley Jordan, Andrew Klein

Background We previously reported that IL-6 blockage by anti-IL6R antibody (mMR16-1) resulted in attenuation of donor-specific antibody (DSA) recall responses in a mouse model of allo-sensitization. However, the mechanism(s) responsible for antibody suppression is poorly understood. Methods C57BL/6 mice were pre-sensitized with skin allograft (SG) from a HLA.A2 transgenic mouse. At Day 70 the pre-sensitized mice were re-stimulated with a second HLA.A2+ skin graft. Recall alloantibody responses were monitored weekly for 4 weeks by measurement of serum anti-HLA.A2 antibodies. T, B and plasma cells were analyzed in FACS and ELISpot assay. Results All the pre-sensitized mice had moderate levels (113.9-143.9 MFI) of DSA IgG in sera before 2nd skin grafting. Re-sensitization in the control group (n=6) resulted in a recall response featured by a surge of anti-HLA.A2 IgG at day 14 (633+52 MFI) and a peak at day 28 (827+110 MFI). mMR16-1 significantly reduced DSA IgG levels (336+116 MFI at day 14 and 530+136 MFI at day 28, P=0.003, P=0.15, respectively, as compared to the control). FACS analysis showed that mMR16-1 has no depleting effect on splenic T-cells, B-cells and CD11b+ monocyte/macrophages in the spleens. There was a reduction in CD138+ plasma cell population in the BM. ELISpot assay showed a significant reduction in IgG+ spots in bone marrow cells derived from allo-sensitized mice treated with anti-IL6R as compared to that of isotype control mice (p=6.15711E-5). A decrease in IgG+ spot formation in the splenocytes was seen in the treated mice. However, the difference in IgG+ spot counts between the treated and the isotype control mice was not statistically significant (p=0.069). Conclusion The data indicate that anti-IL6R antibody may affect the final stage of B cell maturation into plasma cells, or suppression of antibody forming plasma cells during the development of recall alloantibody responses. Thus, one of the mechanisms by which mMR16-1 attenuates alloantibody responses is consistent with a blockage of a described IL-6 activity as a B/plasma cell growth factor. Future studies are directed to mechanistically illustrate how IL-6R blockage suppresses the long-lived plasma cell compartment, memory B and T cells.

Ly6C surface expression marks lineage commitment by mouse granulocyte progenitors (GPs) and monocyte progenitors (MPs)

Alberto Yanez Boyer, Helen Goodridge

Accurate identification of specific progenitors is essential for mapping cell fate choices at the single cell level and for precise definition of mechanisms that underlie hematopoietic cell production from embryogenesis to adulthood, in the steady-state, during an emergency response (infection/inflammation), and in leukemogenesis. Models of hematopoiesis are currently undergoing extensive revision to incorporate newly discovered progenitor populations and hematopoietic lineage maps remain controversial. In the current study we focused on the production of granulocytes (neutrophils) and monocytes/macrophages. Granulocyte-monocyte progenitors (GMPs), which have the potential to produce both granulocytic and monocytic cells, are contained in the Lin- c-Kit+ Sca-1- (LKS-) CD34+ FcgRhi subset of mouse bone marrow. However, not all LKS- CD34+ FcgRhi cells have the potential to produce both granulocytes and monocytes. Only about 40% of colonies formed in methylcellulose under permissive conditions contain both cell types; the rest are either pure granulocyte or pure monocyte colonies, indicating the presence of large numbers of lineage-committed granulocyte progenitors (GPs) and monocyte progenitors (MPs) in the LKS- CD34+ FcgRhi fraction. Using in vitro and in vivo assays, we now show that initiation of Ly6C surface expression marks lineage commitment by these progenitors and can be used to separate “true” oligopotent GMPs from lineage-committed GPs and MPs. This has permitted us to more precisely study the molecular mechanisms that control granulocyte versus monocyte cell fate choice under a variety of conditions, including in response to Listeria monocytogenes infection, which is characterized by enhanced monocyte production. Our ongoing studies are also providing further mechanistic insight into the specification of granulocytic v. monocytic fate at the level of transcription factor expression.

Neurology/Neuroscience

Mutant SOD1 astrocytes display an accelerated aging phenotype in amyotrophic lateral sclerosis

Melanie Das, Clive Svendsen

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized primarily by the death of motor neurons in the brain and spinal cord. However, ALS is not a cell autonomous disease; the glial microenvironment can significantly modulate motor neuron survival. Astrocytes with an ALS causing mutation such as in the protein superoxide dismutase I (SOD1) are detrimental to motor neuron survival. While many have embarked on the quest for the mechanism through which astrocyte-mediated toxicity occurs, the answer remains elusive. The prevalence of amyotrophic lateral sclerosis (ALS) increases with age. However, the possible interaction between the normal degenerative aging process and the rapid cell loss in ALS has not been thoroughly investigated. Here, we find that compared to astrocytes from young rats, astrocytes from aged wildtype rats have a reduced ability to support motor neurons in culture. To investigate an explanation for age-related differences between astrocytes we look at cell senescence. Cellular senescence is a consequence of aging in many tissue types and the accumulation of these non-proliferating cells leads to tissue atrophy. We observe that aged astrocytes contain a higher proportion of senescent cells relative to young astrocytes. Surprisingly, we find that astrocytes from an ALS rat develop a large population of cells in a senescent state at younger age than wildtype rats. ALS astrocytes display a significantly higher proportion of senescent cells compared to an age-matched wildtype counterpart. These data suggest that a mechanism long associated with aging is amplified in the presence of toxic protein stress. Identifying the cellular changes during aging that influence neurodegenerative disease can help us gain insight into identifying new targets to combat ALS.

Murine models of multiple sclerosis and feasibility of clinical translational research

Oana Dumitrascu, Nancy Sicotte, Homayon Ghiasi

Purpose: to compare two animal models of multiple sclerosis (viral-induced CNS demyelination and experimental autoimmune encephalomyelitis) using clinical and hysto-pathologic data and to test potential therapeutic applications for human disease. Methods: C57Bl/6 female mice immunized with MOG, MBP and PLP and ocularly infected mice with a recombinant HSV-1 over expressing IL2 were followed prospectively and scored for clinical signs of MS. Day of onset, clinical score, symptoms incidence and mortality were analyzed. On day 29 post-immunization/ infection, animals were euthanized and brains, optic nerves and spinal cords were harvested. CNS tissues were analyzed for demyelination, inflammation and immune cell infiltration. Clinical behaviour and demyelination were compared between untreated mice and mice treated with various immune-modulatory agents. Results: MOG-injected mice represented the only symptomatic group, with clinical correspondence of relapsing-remitting MS. Optic neuritis was present in the recombinant viral-induced disease and absent in EAE

model. HSV1-IL2-induced disease was characterized by extensive CNS demyelination and inflammation, with good therapeutic response to IL12p70 and IFN-beta cDNA intramuscular therapy. Conclusions and significance: EAE model had no evidence of optic neuritis. HSV-IL2-induced CNS demyelination represents a good model for testing further therapeutic agents. Adequate preclinical data are imperative to prevent clinical trials' failure.

Sleep-disordered breathing and risk for Alzheimer's disease in cognitively normal elderly

Emmanuel During, Ricardo Osorio

Alzheimer's disease (AD) is the most common form of dementia, and is characterized by amyloid beta (Aβ) plaques, neurofibrillary tangles (NFT) resulting in decreased cerebrospinal fluid (CSF) levels of Aβ42, and increases in phosphorylated-tau (P-Tau), total-tau (T-Tau) and P-Tau/Aβ42 ratio.Studies have shown that there is an increased prevalence of sleep disordered breathing (SDB) in AD patients and that SDB and the ApoE4 allele may have additive effects.We performed home monitoring sleep study, measured CSF biomarkers and performed cognitive evaluation on 95 cognitively normal elderly volunteers.25 were considered controls (NL) due to an Apnea Hypopnea Index 4% <5, 51 mild SDB (AHI4% 5-15), and 19 moderated to severe SDB (AHI4%>15). There were no differences across NL and SDB groups in any of the cognitive tests. Analyzing the group as a whole, CSF levels of Aβ42, P-Tau, T-Tau and P-Tau/Aβ42 ratio were not statistically different between the three sleep groups and linear regression analyses showed no association between AHI4% and AD-biomarkers. However, ApoE3 carriers showed significant differences between sleep groups for P-Tau ($F=4.3$, $p<0.025$), T-Tau ($F=3.6$, $p<0.05$) and a marginal difference in Aβ42 ($F=2.9$, $P<0.1$), while, as a whole, AHI4% was a significant positive predictor of P-tau ($\beta=0.3$, $SE=3.0$, $p<0.025$), T-tau ($\beta=0.3$, $SE=21.8$, $p<0.025$) and Aβ42 ($\beta=0.4$, $SE=39.7$, $p<0.01$). In ApoE4+ subjects, severity of SDB trended towards lower CSF Aβ42 levels although there were no significant SDB group differences for Aβ42, T-Tau or P-Tau probably due to lack of power.This could be the first report of an association between intermittent hypoxia and increases in both T-Tau and P-Tau in cognitively normal ApoE3 carrier elderly. Our findings could in fact be showing very early Aβ42 increases before the consequential decreases in Aβ42 that follow amyloid plaque formation.The other possibility is that SDB has a specific anoxic effect on the Hippocampus in ApoE3+ normal elderly that increases P-Tau but does not increase brain amyloid load and therefore is not a direct cause of Alzheimer's as both plaques and NFT are required for the pathological diagnosis of AD. Longitudinal studies would be needed to test these opposing hypotheses.

Paraneoplastic Brainstem Syndrome with Trismus Laryngospasm and Myoclonus in Mixed Mucinous Breast Carcinoma: a case report and review of literature

Joanna Gan, Nancy Sicotte

OBJECTIVE: Cases of paraneoplastic syndrome involving jaw dystonia, laryngospasm are beginning to be described in association with Anti-Ri antibodies. BACKGROUND: These cases have been associated with high morbidity and mortality. (Pittock 2010). Brainstem syndromes, in particular, opsoclonus-myoclonus, have been reported in literature as associated with anti-Ri antibody and breast carcinoma. METHODS: Case report and literature review. RESULTS: We present the first case report of a paraneoplastic brainstem syndrome consisting of a combination of trismus, laryngospasm, and myoclonus that prompted a work-up eventually revealing a mixed mucinous breast carcinoma. Respiratory failure can be a real threat. For identified cases, would recommend careful monitoring for need of intubation and mechanical ventilation. Dysphagia can be severe and a feeding tube may be necessary for adequate nutrition. Plasmapheresis appears to be a helpful treatment modality. CONCLUSIONS: The relationship between mucinous breast cancer and paraneoplastic syndromes have not been established and should prompt further investigation.

Prevalence of non-motor symptoms in Parkinson's disease: A systematic review

Janelle Haider, Michele Tagliati

OBJECTIVE: Aim of this study was to systematically identify non-motor symptoms (NMS) prevalence in patients with Parkinson's disease (PD), as reported in published studies using validated tools. BACKGROUND: NMS are a key determinant of health, quality of life and societal cost in PD patients. Despite their impact, they are not well recognized and their prevalence varies widely among studies owing to varying methodology. METHODS: MEDLINE, Web of Science, CENTRAL and Scopus databases were searched until August 2013. Cumulative plots of prevalence estimates weighted by sample size were created with random effect analysis and the influence of selected key variables, including gender, motor subtypes (tremor-predominant vs akinetic-rigid) and age at onset (<50 vs ≥50 yrs) was examined. RESULTS: From 15726 references identified and screened, 71 were assessed for eligibility and 24 articles, presenting prevalence rates of NMSs based on NMS questionnaire (NMSQuest) or NMS scale (NMSS), were included for a total of 6,378 patients. Multiple publications from the same database were excluded. Nocturia (59.7%), urinary urgency (54.6%), depression (51.7%), constipation (48.5%), anxiety (46.9%), forgetfulness (45.5%) and insomnia (44.7%) were among the most prevalent NMSs, with highly significant statistical heterogeneity. Based on combined prevalence estimates, we found no significant effect of gender, motor subtype or age at onset. CONCLUSIONS: Our study confirms a significant clinical heterogeneity among different studies. Such variation could be in part explained by differences in motor disease severity, although NMS appears to be a quality of life determinant independent of motor state as recently shown in two large controlled studies in untreated and early patients and a clustered study addressing NMS grading.

Age-related CD8+ T cell clonal expansions infiltrate brain and induce neurodegenerative pathology similar to sporadic Alzheimer's Disease

Michelle Jhun, Akanksha Panwar, Altan Rentsendorj, Ryan Cordner, Nicole Yeager, Gretchen Duvall, David Golchian, Armen Mardiros, Yasuko Hirakawa, Keith Black, Christopher Wheeler

Introduction: Alzheimer's Disease (AD), the most common form of age-related dementia, currently afflicts 5.4 million individuals domestically, and is projected to afflict 15 million by mid-century. Patients with mutations in genes that promote toxic amyloid beta (Ab) accumulation in brain develop rare familial forms of AD. Experimental treatments have thus aimed to curtail toxic Ab accumulation, but this approach has been clinically disappointing. One reason may be that >90% of AD patients have sporadic rather than familial AD, and the two may begin and progress differently. Recent evidence suggests that age-related immune defects impact sporadic AD, but this has not been rigorously tested. Clonal expansions of CD8+ T cells (TCEs) represent the most common age-related immune defect. CD8+ T cells can be induced to expand by injecting them into T cell-deficient mice, but their relationship to age-related TCEs and neurodegeneration remain unknown. Methods: We injected purified wild-type, IFNg-deficient, or Perforin-deficient donor T cells into young (6-8 wk) wild-type or T cell-deficient B6.Foxn1 mice and characterized donor cell expansions using age-related TCE markers, as well as pathologic and cognitive hallmarks of AD. Behavioral tests were performed on cell-injected and age-matched control cohorts at various times post-injection (Open Field, 12 wks, 6 and 15 months; Fear Conditioning, 6 mos; Y-maze/Spontaneous Alternation, 10 months; Barnes Maze, 15 months). T cell infiltration and Ab accumulation in brain was assessed early and late, along with astrogliosis, plaque formation, neuronal/synaptic marker levels, and brain mass. Results: CD8+ cells from all donors expanded in B6.Foxn1 hosts 3-4 wk post-injection, and the resulting donor cells were phenotypically similar to age-related TCEs (up-regulated CD122, CD127 & KLRG1; oligoclonal TCR Vb; down-regulated CD8). Donor CD8+ and Ab accumulation in brain were simultaneously detected by Western blot in B6.Foxn1 brains 3 weeks after injection. No difference in Open Field activity of CD8+-injected relative to age-matched controls was detected at any time point. Fear Conditioning indicated low responsiveness of CD8+-injected relative to CD4+- or control-injected B6.Foxn1 6 months after injection, and persistent memory deficits were detected at 10 months by Y-maze. Profound hippocampal learning defects were detected by Barnes Maze at 15 months. Significant accumulation of soluble Ab1-40, immature Ab plaques, neuroinflammation, and Gallyas+ neurofibrillary structures in hippocampus and cingulate cortex were seen in CD8+-injected B6.Foxn1 brains by 15 months, together with loss of neuronal markers and progressive cerebral atrophy (5 and 10% at 6 and 15 months, respectively). Notably, B6.Foxn1 mice treated with Perforin-deficient CD8+ T cells exhibited no neuropathology, and lacked cognitive deficits. In contrast, B6.Foxn1 mice treated with IFNg-deficient CD8+ T cells were largely cognitively intact and exhibited elevated neuronal markers and brain weight, yet harbored hippocampal plaques and Gallyas+ neurons. Conclusions: CD8+ T cell expansions identical to age-related TCEs are readily induced in young B6.Foxn1 mice, where they enter the brain to cause function-dependent neurodegenerative and cognitive pathology. Notably, lytic and pro-inflammatory CD8+ T cell functions may independently impact development of this neuropathology. This non-transgenic model uniquely recapitulates multiple

aspects of sporadic AD and may thus be useful in clarifying the etiology, biology, and treatment of this disease.

Disruption of DYRK1A is Associated with Primary Microcephaly, Intellectual Disability, and Epilepsy

Jianling Ji, Naghmeh Dorrani, John Mann, Hane Lee, Joshua Deignan, Natalie Gallant, Eric Vilain, Wayne Grody, Stanely Nelson, Fabiola Quintero-Rivera

DYRK1A (dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A) is a highly conserved gene located in the Down syndrome critical region, which is responsible for the majority of phenotypic features in this syndrome. It plays an important role in controlling brain growth through neuronal proliferation and neurogenesis. Microdeletions of chromosome region 21q22.11q22.3 that involve DYRK1A contribute to a spectrum of neurodevelopmental phenotypes; however, the impact of DYRK1A disruption has not been fully explored. To characterize the structural variation landscape of DYRK1A disruptions and the associated phenotype, we identified three individuals with de novo genomic disruption of DRYR1A (a 3.6 Mb microdeletion of 21q22.13q22.2, a nonsense mutation c.312C>G p.Tyr104 * and a missense mutation c.563 A>T p.Lys188Ile) through cytogenomic microarray and exome sequencing, respectively. All three patients exhibited microcephaly (OFC <3%, ≤2SD), intellectual disability, and developmental delay. We also performed genotype-phenotype analysis on all reported cases with disruption of the DYRK1A gene via 21q22.11q22.3 microdeletions, intragenic deletions, point mutations and translocations. The genomic impact ranged from a single base pair mutation to a 5.4 Mb deletion. Our analysis revealed that phenotypes were largely indistinguishable between patients with the 21q22.11q22.3 microdeletion and those involving only DYRK1A. All patients exhibited microcephaly (OFC <3%), intellectual disability and developmental delay. Epilepsy was present in 3/4 of all patients. Similar phenotypes have been observed in a Dyrk1a (+/-) deficient mouse model, showing that Dyrk1a expression alters different cellular processes during brain development. Patients with intragenic deletions (52~69kb) showed overlapping phenotypes, highlighting the limitation of routine clinical microarray assessments using stringent cutoffs (>100Kb). Although we cannot exclude contribution of other genes in patients with a microdeletion, the involvement of DYRK1A is sufficient for the clinical manifestation of neurodevelopmental abnormalities. Our study further demonstrates that haploinsufficiency of DYRK1A results in microcephaly and intellectual disability in humans. DYRK1A disruption should be considered in patients with primary microcephaly.

Targeted ACE overexpression in monocytes diminishes Alzheimer's-like pathology and prevents cognitive decline

Yosef Koronyo, Sebastien Fuchs, Julia Sheyn, Brenda Salumbides, Ellen Bernstein, Dieu-Trang Fuchs, Keith Black, Xiao Shen, Kenneth Bernstein, Maya Koronyo-Hamaoui

Our studies indicated a fundamental role for monocyte-derived macrophages (MF) in facilitating clearance of amyloid β-protein (Aβ) and attenuating Alzheimer's (AD)-like cognitive decline. Angiotensin-converting enzyme (ACE) is known to degrade neurotoxic Aβ1-42. Mice overexpressing ACE in myelomonocytes (under the c-fms promoter; called ACE10 mice), demonstrate enhanced innate immune responses. To assess effects of ACE10 on AD, we crossed APPSWE/ΔE9 transgenic (AD+) mice with ACE10 mice. To assess the therapeutic role of ACE10 monocytes, we studied the effects of adoptively transferred ACE10 vs. WT monocytes into the peripheral blood of AD+ mice. Evaluation of brains from AD+ mice with one or two ACE10 alleles demonstrated a drastic reduction in soluble and insoluble Aβ1-42, plaque burden, and astrogliosis. Inhibition of ACE-catalytic domains eliminated this cerebral Aβ reduction in AD+ACE10 mice. Endothelial ACE expression was found essential for perivascular Aβ deposition. There were increased ACE-overexpressing MΦ abundant surrounding and engulfing Aβ plaques. At 11 and 12 months, AD+ACE10 mice were essentially equivalent to non-AD mice in cognitive performance. Moreover, adoptive transfer of ACE10 vs. WT monocytes to AD+ mice was particularly effective in preventing cognitive decline. Our data demonstrate that ACE10 genotype leads to a striking attenuation of AD-like progression in murine models.

Intra-arterial Delivery of Neural Progenitor Cells after Focal Ischemia in the Adult Rat

I-Farn Lei, Jessica Lamb, Padmash Rajput, Lifu Zhao, Jilin Bai, Genevieve Gowing, Clive Svendsen, Patrick Lyden

Utilizing a minimally invasive approach for stem cell transplantation offers a novel treatment strategy for stroke patients. Here we transplanted rat neural progenitor cells (rNPCs) into the stroke brain using this approach. We hypothesized that entrance of intra-arterially infused rNPCs rely upon blood brain barrier (BBB) permeability after stroke. Stroke was induced by middle cerebral artery occlusion with a suture for 2 hours in adult, Sprague-Dawley rats (N=16). rNPCs were infused through an intra-arterial catheter at various time points after 2 hours of MCAo. Two weeks after cell transplantation, we performed immunostaining to identify rNPCs in the ischemic hemisphere. We observed transplanted neural progenitor cells in the ischemic hemisphere at 4 hours, 6 hours, 3 days, and 7 days after MCAo. Furthermore, we detected rNPCs away from the lesion, migrating to a different area of the ischemic hemisphere. This preliminary finding suggests intra-arterially infused rNPCs localized to the lesioned and pan-necrotic site at acute and extended time points after MCAo.

Microglia, a new cellular factor, in the development of hypertension

You Li, Liang Li, Kenneth Bernstein, Xiao Shen, Peng Shi

The concept of neuroinflammation in the development of hypertension has been acknowledged recently, as studies showed that anti-inflammation treatments attenuated Ang II induced blood pressure increase. Microglia are the major immune cells in the central nervous system. To examine the role of microglia in hypertension, we applied a microglial depletion strategy by using a transgenic mouse line CD11b-DTR. This mouse line has an inserted transgene coding simian diphtheria toxin receptor (DTR) under the control of the CD11b promoter. CD11b-DTR mice were made hypertensive by subcutaneous Ang II infusion. Two weeks later, the mice received intracerebroventricular infusion of either DT or saline. Blood pressure was monitored by tailcuff. It showed that systolic blood pressure reached ~140 mmHg in both groups by 2 wk. Microglial depletion caused a gradual reduction in blood pressure (20 mmHg) by the end of 4 wk. To validate the importance of microglia in neuroinflammation associated with hypertension, the expression of pro-inflammatory cytokines in paraventricular nucleus (PVN) were analyzed by quantative RT-PCR. Microglial depletion reduced the expression of both IL-1β and TNFα in the PVN of hypertensive mice. Moreover, the norepinephrine levels in the kidney were significantly decreased in the microglia-depleted mice vs. controls. These data strongly support our hypothesis that in hypertension, microglia play an importance role in initiating neuroinflammation and in sustaining blood pressure.

Biomechanical Analysis of Direct Lateral Interbody Fusion (DLIF) Strategies for Adjacent Segment Degeneration (ASD) in the Lumbar Spine

Melodie Metzger, Ruben Maldonado, Mark Svet, Frank Acosta

Background: Spine surgeons are increasingly faced with a growing number of patients presenting symptomatic ASD after posterior spinal fusion. This typically requires a second surgical procedure to extend the fusion by removing and replacing the existing hardware. DLIF technology may provide a less-invasive alternative; however, no research has been conducted to determine if it provides adequate stability. Methods: Human cadaveric lumbar spines were tested, first intact, followed by 2-level fusion and then extension of the fusion to a third-level using DLIF with and without supplemental hardware followed by the standard posterior approach. Specimens were non-destructively loaded in all planes of motion while three-dimensional kinematics were recorded. Results: Average range of motion at the add-on level was reduced with both DLIF and standard techniques, most significantly with pedicle screws ($p<0.05$). DLIF with added lateral plate significantly stabilized the segment in lateral bending and torsion, while DLIF with cortical screws provided significant stability in flexion/extension and lateral bending. Discussion: Data generated from the study indicate that DLIF add-on techniques with posterior or lateral supplemental fixation may provide adequate stabilization, comparable to the current standard of care. These results provide a biomechanical rationale for the consideration of minimally-invasive options for treatment of ASD.

Suspected TIA using an Expedited Pathway (STEP)

Mani Nezhad, Mohammad Shafie, Shlee Song, Jeanne Black, Tingjian Yan, Laurie Paletz, Sonia Guerra, Patrick Lyden

Background - Prior studies have shown urgent evaluation of patients with suspected TIA leads to a reduction in cost and morbidity. However, the optimal setting for urgent evaluation has yet to be established. Objective - To determine the effectiveness of an expedited testing pathway in reducing length of stay (LOS) and direct costs in individuals with suspected TIA Methods - A prospective observational before-and-after study design was used in two phases. Initially patients (Aug 2011 - Nov 2012) presenting to the Emergency Department (ED) with suspected TIA or suspected stroke with resolving symptoms underwent expedited testing; later (Dec 2012 - June 2013) this was expanded to include patients presenting to the ED, referred by physician, or already hospitalized patients. Using an integrated multidisciplinary team the expedited pathway included evaluation by a neurologist, imaging of the head and neck vasculature, lipid panel, A1c, depression screen, and stroke education to be completed within 23 hours. If indicated echocardiogram and therapy evaluation were also done. Primary endpoints included LOS and direct cost. Results - A total of 399 patients evaluated for suspected TIA; 316 in the intervention group, and 86 in the comparison group. The mean age of the total sample was 72 years (SD=14.6). Demographics of the sample revealed 53% female, 70% Caucasian, 5% Hispanic. Medicare was the primary insurer for 50% of the sample. A statistically significant difference in frequency of observation admissions was seen in the comparison (21.4%) versus the intervention group (18.0%) ($p=0.048$). The unadjusted LOS in the intervention group was 0.34 days shorter than the comparison group, and the unadjusted mean direct cost was $371 less than the comparison group. These differences showed significance using a Wilcoxon-Mann-Whitney test ($p<0.5$). Conclusion - Implementing an expedited testing pathway for suspected TIA shows a significant decrease in LOS and overall costs despite pre-existing co-morbidities.

Glatiramer acetate induces cerebral infiltration of osteopontin-expressing monocytes that facilitate Aβ clearance

Altan Rentsendorj, Brenda Salumbides, Julia Sheyn, Yosef Koronyo, Sandrine Fuchs, Keith Black, Maya Koronyo

Osteopontin (OPN) is a glycoprotein highly expressed by BM-derived monocytic cells that serves as an immune modulator of macrophage function at multiple levels. Our previous data indicated that attenuation of AD-like progression via GA immunization is, at least in part, due to enhanced BM-monocyte cerebral infiltration. To understand the role of OPN, its expression was studied in GA-immunized APPSWE/PS1dE9 mice and in primary BM-derived cultures in vitro. We found distinctive elevated OPN expression patterns in different brain regions associated with reduced Alzheimer's disease pathology following GA immunization and combined treatment of GA with i.v. infusion of BM-

monocytes. OPN is found surrounding Aβ plaque sites and OPN colocalized with, and around Iba1+CD45high macrophages. Correlogram analysis of quantitative immunohistochemical data from these brain regions indicated a significant linear association between OPN and infiltrating monocytes, as well as inverse relationship between OPN levels and Aβ plaque burden. In vitro analysis, using ELISA, Western blotting and immunocytochemical assays, revealed that GA modulates OPN expression in BM-macrophages concomitant to enhancing cellular uptake of fibrillar Aβ 1-42. These in vitro results mirror our in vivo data reinforcing the notion that OPN mediates migration of BM-monocytes to the diseased brain and increase macrophage Aβ1-42 uptake.

Regenerative Medicine/Stem Cells

Heterotypic cellular reprogramming to corticofugal neuronal subtypes using a novel genetic system for inducible expression of Fezf2

Aslam Akhtar, Gi Bum Kim, Jessica Molina Aravena, Joshua Breunig

The neurons of the cerebral cortex arise from neural stem cells during embryogenesis to generate a six layer structure. These cells terminally differentiate into precise subtypes of neurons. The intrinsic and extrinsic determinants of cerebral cortex development and the establishment of laminar neuronal subtype identities are increasingly understood. In particular, the transcription factor Fezf2 has been identified as a critical determinant of layer 5 corticofugal projection neurons. This class of neurons includes corticospinal motor neurons that are lost in degenerative motor neuron disorders such as amyotrophic lateral sclerosis (ALS). Previous work has explored the ability of Fezf2 to reprogram cells to a corticofugal phenotype within a very spatial and temporal window of early development. We have developed an inducible and reversible, 3rd generation, doxycycline(Dox)-regulated genetic system for expressing Fezf2. When our expression vector is induced by Dox in postnatal mouse neural stem cells or astrocytes, Fezf2 causes morphological conversion into neuron-like cells despite the presence of growth factors and serum. We have verified that this system is very robust upon in vivo electroporation, displaying little or no leakage and excellent inducibility. Using this new technology, we are exploring the ability of Fezf2 to reprogram heterogenous populations of stem, progenitor and terminally differentiated cells to corticofugal subtypes in postnatal and adult mice, including after cortical lesion and in neurodegenerative disease models. Further, we will explore the suitability of this tool for generating enriched populations of motor neurons for studying disease processes in patient-derived iPS cells.

Therapeutic efficacy of cardiosphere-derived cells in a transgenic mouse model of nonischemic dilated cardiomyopathy

Mohammad Amin Aminzadeh, Eleni Tseliou, Baiming Sun, Ke Cheng, Konstantinos Malliaras, Raj R Makkar, Eduardo Marban

Aims: Cardiosphere-derived cells (CDCs) produce regenerative effects in the post-infarct setting. However, it is unclear whether CDCs are beneficial in non-ischemic dilated cardiomyopathy (DCM). We tested the effects of CDC transplantation in mice with cardiac-specific Gαq overexpression, which predictably develop progressive cardiac dilation and failure, with accelerated mortality. Methods and Results: Wild-type mouse CDCs (105 cells) or vehicle only were injected intramyocardially in 6-, 8- and 11-week old Gαq mice. Cardiac function deteriorated in vehicle-treated mice over 3 months of follow-up, accompanied by oxidative stress, inflammation and adverse ventricular remodeling. In contrast, CDCs preserved cardiac function and volumes, improved survival, and promoted cardiomyogenesis while

blunting Gαq-induced oxidative stress and inflammation in the heart. The mechanism of benefit is indirect, as long-term engraftment of transplanted cells is vanishingly low.Conclusions: CDCs reverse fundamental abnormalities in cell signaling, prevent adverse remodeling and improve survival in a mouse model of DCM. The ability to impact favorably on disease progression in nonischemic heart failure heralds new potential therapeutic applications of CDCs.

Development of a "disease in a dish model" of Inflammatory Bowel Disease using human intestinal organoids

Robert Barrett

Inflammatory Bowel Disease (IBD) is a chronic inflammatory disorder that affects the gastrointestinal tract. Despite extensive research, the causes of IBD remain elusive. One aspect that has been poorly studied is the role of the intestinal epithelium in this disease. Interestingly, genome wide association studies have revealed that SNPs in intestinal epithelial genes are associated with IBD. As numerous repositories worldwide contain genotyped EBV B-cell lines that possess these SNPs, the aim of this study was to develop a disease in a dish model of IBD whereby human intestinal epithelium containing these SNPs could be generated. To establish this method, control EBV B-cell lines were reprogrammed to form IPSCs. These IPSCs were then directed to form intestinal organoids that contain all the differentiated intestinal cell subtypes. This will permit the generation of intestinal epithelium containing IBD associated SNPs and potentially allow an assessment of the functional consequences of such SNPs.

iPS cells can be efficiently differentiated back to MSCs using a short exposure to TGFβ

Shiran Ben-David, Susan Su, Dmitriy Sheyn, Dan Gazit, Zulma Gazit

Mesenchymal stem cells are the ultimate source for tissue engineering of skeletal tissues and regeneration of various skeletal conditions. However, their availability and self renewal ability is limited. Moreover, osteoporotic patients have decreased numbers of MSCs and these are dysfunctional. The recent discoveries of the differentiated cells reprogramming opens new horizons in stem cell therapy and may be employed to overcome these challenges. We hypothesized that iPSCs differentiated back to MSC-like cell that can be used for bone formation. The cells were directed towards mesenchymal lineages using short-term exposure of the embryonic bodies to TGFβ. The transition of the attached cells towards MSC stage was documented using FACS analysis against mesenchymal markers and was much more efficient than passive differentiation and outgrowth of MSCs from the embryonic bodies. Differentiation potential towards the three mesenchymal lineages was evaluated in vitro. Bone formation in vivo was achieved using transient overexpression of BMP6 and ectopic implantation. Additionally we performed RNA sequencing of the new stem cell populations and compared to the bone marrow derived MSCs. We could found that the MSCs derived from iPS cells, are more advanced in their differentiation stage than bone marrow MSCs, but comparably competent for bone tissue formation.

Lineage Tracing of Oval Cell Population During hepatic Cell Differentiation

Deisy Contreras, Joseph Irudayam, Aparna Subramanian, Vaithi Arumugaswami

Liver has higher regenerative capacity compared to other vital organs. Liver stem or progenitor cell population resides in the portal triad. Upon acute and chronic liver injury, the liver progenitor cells expand and replace the injured hepatocytes. The liver progenitor cells are called oval cells based on morphology and molecular marker expression. We hypothesize that hepatic differentiation of pluripotent stem cells recapitulates the hepatic progenitor oval cell population. First we looked at lineage tracing of oval cell population during hepatic cell differentiation using molecular markers CD34 and CD90. We used a three stage hepatic differentiation protocol comprised of generation of definitive endoderm cells (day 5), hepatic lineage specification (days 6 to 15) and hepatic maturation (days 16-21). We observed that during the early stages of differentiation (days 6 to 15) the oval cell population is present at 2-3% level but gradually decreases as differentiating towards a more mature hepatocyte-like cell population (day 21). To conclude, during hepatic differentiation of human pluripotent stem cells, we have identified CD34, CD90 double positive oval cell population. Currently, we are investigating the signaling pathways and molecular signatures specific for oval cell population as well as functional analysis of the oval cell versus non-oval cell population in terms of mature hepatocyte yield.

Systemic treatment for multiple lumbar bone defects using combined human MSCs and PTH therapy

Xioayu Da, Dmitriy Sheyn, Wafa Tawackoli, Deuk Jun, Young Koh, Hyun Bae, Susan Su, ZulmaGazit, Gadi Pelled, Dan Gazit

Vertebral fractures are the most common fractures associated with osteoporosis. We hypothesized that combined administration of MSCs and PTH would induce stem cell homing to vertebral defects followed by osteogenesis and repair. We induced osteopenia in rats by ovarioectomy and low calcium diet that resulted in 45-50% loss of bone mineral density. Human BM-MSCs were labeled with Luciferase or NIS reporter genes. Multiple defects were created in the lumber spine and treated with IV injection of cells and daily SQ injections of PTH or saline. Cell survival and homing to the defect site were tracked using BLI and immunohistochemistry or μSPECT/μCT. The defect repair was monitored using μCT. Vertebral defects treated with the combined MSC-and-PTH therapy resulted in 2-fold increase in bone volume density when compared to defects treated with PTH only. Our results show that vertebral defects in osteopenic rats were repaired significantly more efficiently when treated with MSCs and PTH, compared to the controls. Moreover, when we tracked labeled MSCs using optical and nuclear imaging systems, we could detect cell homing to the lumbar region. This study provided evidence for future therapies that could revolutionize the treatment of vertebral and other complex fractures especially in osteoporotic patients.

Cardioprotection by cardiosphere-derived cells administered 20 min after reperfusion in rats: reductions of infarct size, apoptosis, and macrophage infiltration, accompanied by improved function

Geoffrey de Couto, Eleni Tseliou, Hideaki Kanazawa, Eduardo Marban

Introduction: Cardiosphere-Derived Cells (CDCs) have been shown to reduce scar size following an established myocardial infarction (CADUCEUS Trial). It is currently unknown whether CDCs confer acute cardioprotection following ischemic injury. Here we test the hypothesis that intracoronary infusion of CDCs following ischemia/reperfusion (I/R) injury reduces cardiomyocyte stress, and protects against cardiac dysfunction and infarct expansion. Methods & Results: Wistar-Kyoto rats (aged 8-12 weeks) underwent 45 minutes of ischemia and 20 minutes of reperfusion. Animals were then randomly allocated to either CDC or placebo groups and treated with 5x105 CDCs or PBS, respectively. Two days following I-R injury, CDC-treated rats revealed a reduction in percent infarct mass relative to PBS-treated controls (6.3% vs. 13.6%, $p<0.01$ n=5/group). These findings were accompanied with reduced programmed cell death within the infarct zone at day 2 (cleaved caspase 3, TUNEL positivity; $p<0.05$), and improved cardiac function at day 14 (EF: 45% vs. 62% $p<0.01$). While no difference was observed in peripherally circulating inflammatory cells, a reduction in CD68+ macrophages (Mf) was found within the myocardium of CDC-treated animals (23% vs. 18%, $p<0.05$; n=4/group). In vitro co-culture of CDCs with TNF-a-stimulated Mf cells attenuated the expression of IL-6, while concomitantly elevating IL-10, Tgfb1, and Arg1, gene expression profile within Mf cells ($p<0.05$). Conclusions: Collectively, these data demonstrate that intracoronary infusion of CDCs following I/R produce acute cardioprotection, and confer prolonged myocardial integrity. Furthermore, CDCs reduce infarct expansion at 2 days and improve cardiac function at 14 days. The cardioprotective effects of CDCs involve a reduction in infiltrating, cytotoxic Mf cells.

Differentiation strategy for neural crest stem cell (NCSCs) and Schwann cell precursor (SCPs) production from human induced pluripotent stem cells (iPSCs) derived from Charcot-Marie-Tooth (CMT) patients

Irina Epifantseva, Kevin Kim, Megan Simpkinson, Sharon Cormona, Shaughn Bell, Ghulam Muhammad, Dhruv Sareen, Robert Baloh

Charcot-Marie-Tooth (CMT) is one of the most common inherited disorders of the nervous system, results in altered myelination, degeneration of the longest axons, sensory loss in extremities and neuropathic pain. Currently there are no disease modifying treatments or any regenerative therapies available for this disorder. In order to perform disease modeling and investigate regenerative therapies we differentiated iPSCs (3 controland 3 CMT1A lines) into large maintainable population of neural crest stem cells, and subsequently into a nearly pure population of Schwann cell precursors. Preliminary results showed that differentiated Schwann cells from control iPS lines (but not from CMT1A lines) are functional and myelinated rat dorsal root ganglia and motoneurons in vitro. Ongoing studies involve determining how CMT1A lines differ from controls in their ability to generate myelin in vitro and in vivo,

and whether iPSC derived Schwann cells can promote axonal survival after transplantation into a CMT1A rat model.

Clinical-grade neural progenitor cells secreting GDNF for the treatment of ALS

Genevieve Gowing, Brandon Shelley, Kevin Staggenborg, Jessica Latter, Pablo Avalos, Leslie Garcia, Jessica Zelaya, Clive Svendsen

Human, fetal cortex-derived neural progenitor cells (hNPCs) can be expanded in vitro and survive and integrate into large and small animals following transplantation, such as rat models of ALS. Furthermore, hNPCs can be genetically modified to secrete glial cell line-derived neurotrophic factor (GDNF), a powerful growth factor that has been shown to have a neurotrophic effect in animal models. We have demonstrated that transplanting hNPCs engineered to secrete GDNF can rescue the microenvironment and preserve dying motor neurons in animal models of ALS. Moving towards the clinic, we have generated a GMP-grade master cell bank of hNPCs and sourced it to generate research and clinical-grade cell lots transduced to secrete GDNF with GMP-grade lentivirus. We have completed the dose ranging aim of the project using an ALS rat model and are moving forward with the safety/toxicity aim required by the FDA for approval of a phase 1/2a drug safety trial.

Limited radiation enhances gene targeting in human pluripotent stem cells

Seigo Hatada, Aparna Subramanian, Berhan Mandefro, Songyang Ren, Ho Won Kim, Ji Tang, Vincent Funari, Dhruv Sareen, Vaithilingaraja Arugugaswami, Clive Svendsen

Human pluripotent stem cells (hPSCs) are set to revolutionize both disease modeling and cell therapy. However, efficient gene targeting is crucial to develop isogenic control or reporter lines. Here we show that limited low doses (0.2 to 0.4 Gy) of γ-ray or X-ray exposure induce DNA repair/recombination machinery (ataxia-telangiectasia mutated, Histone H2A.X and RAD51 proteins) and, when combined with recently developed engineered nucleases (such as zinc finger nucleases or clustered regularly interspaced short palindromic repeats), a synergistic effect is observed by substantially increasing the targeting frequency compared with engineered nucleases alone. Irradiated and targeted lines are karyotypically normal, expand efficiently and are able to make all differentiated lineages that continue to express GFP targeted at the AAVS1 locus. This simple, accessible and novel process allows rapid production of targeted hPSC lines.

Cardiosphere-derived cells secrete exosomes containing mirs which stimulate cardiomyocyte proliferation and angiogenesis in vitro, and improve functional recovery after myocardial infarction in mice

Ahmed Ibrahim, Ke Cheng, Eduardo Marban

Background Exosomes are nano-sized bilayer vesicles that are secreted by most cell types. Exosomes are rich in microRNAs (mirs) which may function in a paracrine fashion. Cardiosphere-derived cells (CDCs) have been shown to regenerate heart after myocardial infarction (MI) in animal models and in the CADUCEUS clinical trial. However, most of the regenerated muscle is of innate origin, which suggests that CDCs function mainly through indirect pathways. METHODS and RESULTS In vitro and in vivo, we compared three treatment groups: vehicle only (control), CDC-derived exosomes and normal human dermal fibroblast (NHDF)-derived exosomes. Neonatal rat cardiomyocytes (NRCMs) incubated with CDC exosomes expressed higher levels of Ki67 (CDC: 42.7 ± 0.04%, NHDF: 22.5 ± 0.04%, control: 9.1% ± 0.03, n=4, p<0.001) and lower expression of tunel compared to NHDF exosomes or control (CDC: 25.2 ± 0.04%, NHDF: 45.1 ± 0.05, control: 41.4 ± 0.05%, n=4, p<0.01). CDC exosomes also stimulated tube formation in HUVEC cells compared to NHDF exosomes or control (CDC: 9393 ± 689; NHDF: 2813 ± 494.5; control: 1097 ± 116.1, n=3, p<0.05). SCID mice injected with exosomes from CDCs during acute MI showed higher LVEF at two weeks (CDC: 40.8 ± 2.33 NHDF: 32.34 ± 2.0, control: 31.31 ± 3.2, n=6, p<0.05) and four weeks (CDC: 44.03 ± 1.5 NHDF: 31.8 ± 1.7, control: 31.5 ± 2.7, n=6, p<0.05) post MI, as well as increased viable mass. Mir microarray analysis identified mir-146a, mir 22, mir 24, and mir 210 among the most highly-upregulated mirs in CDC-exosomes compared to NHDF (262, 59, 50, and 30 fold respectively). Mir-146a-treated NRCMs were more resistant to H2O2-induced stress compared to a mimic control. Array analysis of these NRCMs treated also showed suppression of IRAK1 and TRAF6 transcripts (1.9 and 2.0 folds lower than control respectively, n=4). Conclusions Mir-containing exosomes secreted by CDCs exhibit multiple beneficial effects on injured myocardium, suggesting that exosomes may mediate some of the therapeutic effects of CDCs. Most notably mir-146a provides cardioprotection in an acute model of MI.

Organ engineering using decellularized liver scaffold recellularized with iPSC-derived hepatocytes

Vidhya Kanagavel, Songyang Ren, Joseph Irudayam, Weidong Xiong, Dodanim Talavera, Andrew Klein, Samuel French, Vaithi Arumugaswami

Liver transplantation is the option for end-stage liver diseases. Shortage of donor liver organ is a major issue. To overcome this, alternative approaches including, hepatic cell transplantation, bioartificial liver devise and whole liver organ engineering, are being investigated. We focus on developing engineered liver using natural liver scaffold generated via decellularization process. The liver scaffold would maintain the authentic three dimensional micro-architecture and vasculature. The liver scaffold repopulated with patient-specific hepatic cells differentiated from induced pluripotent stem cells (iPSC) is a promising option. For this proof-of-concept, we decellularized rat liver by perfusing detergents SDS and Triton x-100. Histological analysis of decellularized liver matrix (DLM) revealed removal of cellular

component and preservation of extra-cellular architecture. Presence of collagen type IV and laminin in the DLM was verified. Human endothelial cells seeded through DLM portal vein present in the vascular linings. We have also established an efficient protocol for deriving functional hepatic cells from iPSC. Perfusion of DLM with green fluorescent protein labeled iPSC-hepatocytes resulted in recellularization of liver parenchymal space. Currently we are assessing the in situ functions of repopulated iPSC-hepatocytes. In conclusion, we have generated natural liver scaffold from rat that can be populated with human iPSC-hepatocytes and endothelial cells.

Human induced pluripotent stem cells and their utility in studying neuron-muscle interactions in vitro

Lindsay Lenaeus, Berhan Mandefro, Loren Ornelas, Anais Sahabian, Dhruv Sareen

The Cedars-Sinai Regenerative Medicine Institute Induced Pluripotent Stem Cell (iPSC)Core Facility has generated ~70 iPSC lines from a variety of healthy anddiseased individuals. The Core provides fully characterized, multiple clonal iPSC lines generated from each patient. A major function of the Core is to maintain high quality and reliable iPSC lines over long-periods so that our users can effectively model human diseases in a dish. Here we show that we achieved excellent quality iPSC lines by optimizing, 1) passaging densities tailored to each line's growth characteristics, 2) novel defined media, 3) growth substrates, and 4) daily removal of spontaneous differentiation through manual methods, which is a very time-consuming and labor-intensive process. An important application of iPSCs is to createbiological models of disease by differentiating into a variety of cell types pathognomonic to the disease. Using iPSCs we describe here the development of models to recreate human neuromuscular junctions (NMJs) in vitro and determine the relationship of healthy or diseased iPSC-derived motor neurons (iMNs) to healthy fetal skeletal muscle cells (SkMCs). Briefly, we optimized co-culture systems for iMNs and SkMCs by mixing each cell type either on coverslips or maintaining them in separate compartments using Xona Microfluidic devices that allow facile visualization of axonalinteractions with the muscle and formation of NMJs using live imaging and α-bungarotoxinstains. Future studies will investigate formation of NMJs using iMNs from healthy and diseased individuals with known neuromuscular diseases namely,Spinal Muscular Atrophy (SMA) and Amyotrophic Lateral Sclerosis (ALS). We willdetermine the temporal contribution and mechanisms of how mutant iMNs from SMAand ALS individuals may result in malfunctioning NMJs in vitro. We hypothesize that diseased iMNs will be either unableto develop adequate NMJs and/or result in their degradation over time due to deathof mutant MNs.

Generation of an expandable and stable population of human motor neuron precursors in suspension cultures derived from induced pluripotent stem cells

Berhan Mandefro, Loren Ornelas, Lindsay Lenaeus, Dhruv Sareen, Clive Svendsen

Spinal Muscular Atrophy (SMA) and Amyotrophic Lateral Sclerosis (ALS) are a group of progressive neurological disorders that selectively affect motor neurons (MNs) in children and adults, respectively, causing muscle weakness, paralysis and often resulting in death. It remains unclear how MNs are specifically vulnerable to degeneration in both diseases. Increasing MN survival is one of the best predictors of drug success, but these assays have been performed in patient fibroblasts or tumor lines. A new paradigm in disease modeling and drug discovery is the generation of Induced Pluripotent Stem Cells (iPSCs) from affected patients, differentiation and then studying pathophysiologically affected MNs. With High Content Imaging (HCI) we can assay overall cell survival and fiber outgrowth from multiple patient-derived MNs. However, obtaining large numbers of MNs reproducibly is critical. In this study we developed a method to obtain expandable and stable populations of MN precursors in sphere cultures from multiple healthy and patient-iPSCs with the goal of performing HCI compound screens. Non-integrating human iPSCs were harvested with accutase to form uniformly sized neural aggregates (20,000 cells) by centrifugation in 384-well PCR plates. Neural induction was performed in media supplemented with SMAD inhibitors, LDN1931890 and SB431542. Subsequent plating on laminin yielded neural rosettes that were selectively lifted, caudo-ventralized in neural media supplemented with retinoic-acid and purmorphamine, and cultured as iPSC-derived MN precursor spheres (iMNPS) in defined MN media supplemented with EGF and FGF2. Expansion of iMNPs was performed for multiple passages using an automated chopper. Fresh-fixed-frozen iMNPS sections routinely processed for immunostaining revealed that the iMNPS proliferated (Ki67+) and were stable over multiple passages (over 20 weeks) maintaining expression of MN progenitor markers (Nestin, Olig2, Hb9, Islet1, Nkx6.1, Lhx1, LhX3, TuJ1 and SMI32). Importantly, the iMNPs also cryopreserved with excellent post-thaw cell viability (>90%). Withdrawal of mitogens from iMNPS in maturation media resulted in terminal differentiation consistently yielding mature MNs (Map2a/b, ChAT, and SMI32) on purified laminin substrate. MN cell counts, viability and neurite outgrowth was performed by HCI. In conclusion, we have developed a novel and reliable method to generate an expandable population of MN precursors amenable for high-content screening of MNs after plate-down.

Huntington's Disease iPSC-derived striatal cells are specifically susceptible to the loss of BDNF due to increased extrasynaptic glutamate receptors

Virginia Mattis, Colton Tom, Michael Oestergaard, Amber Southwell, Crystal Doty, Loren Ornelas, Anais Sahabian, Dhruv Sareen, Frank Bennett, Michael Hayden, Clive Svendsen

Huntington’s disease (HD) is a fatal autosomal dominant neurodegenerative disorder affecting over 30,000 US citizens, with another 150,000 currently at risk in the US alone. Neuronal destruction results in slow death occurring over 10-25 year period. HD is caused by the expansion of polyglutamine (CAG) repeats in the Huntingtin gene. One theory is that HD pathology results from the lack of brain-derived

neurotropic factor (BDNF) in the cortical-striatal pathway, leading to death of striatal neurons. With the HD iPSC consortium, we have recently reported a new in vitro model of HD based on the generation of induced pluripotent stem cells (iPSCs) from subjects with 60 or 180 CAG repeats and controls. HD and control iPSC lines showed differential risk of cell death, specifically upon BDNF withdrawal or exposure to the excitotoxin glutamate. We therefore wanted to use this novel HD model to study the roles of BDNF in HD and its potential interactions with the glutamate excitotoxicity seen in the disease. We show that upon BDNF withdrawal, the HD cells (180 or 109 CAG repeats) upregulate the extrasynaptic NMDA receptor subunit NR2B. We therefore went on to examine if by blocking glutamate signaling we could reverse the cell death seen in the HD iPSC-derived striatal cells upon BDNF withdrawal. Indeed, cell death can be prevented by not only blocking all glutamate signaling, but importantly also by specifically blocking the extrasynaptic glutamate signaling pathways. Lastly, we also demonstrate that the cell death phenotype can be reversed by targeting the expanded CAG repeat using anti-sense oligonucleotide technology. This study provides important insight into HD mechanisms for future therapeutic targets.

Evidence for a dying forward process of ALS in the SOD1G93A rat

Gretchen Miller, Clive Svendsen

The mechanisms underlying the causality of motor neuron death in Amyotrophic Lateral Sclerosis (ALS) have yet to be elucidated. Conflicting reports exist in support of two main hypotheses: (i) The “dying-forward” hypothesis proposes that ALS is mainly a disorder of cortical motor neurons, which connect with anterior horn cells of the spinal cord mediating anterograde degeneration of these cells leading to muscle degeneration. (ii) The “dying-back” hypothesis proposes that ALS begins at the neuromuscular junction. These hypotheses are largely unexplored in the hSOD1G93A rat model of ALS. Here, we evaluated the degree and timing of degeneration in the cortex, spinal cord, ventral root axons and muscle in pre-symptomatic, early symptomatic and endpoint SOD1 rats to address where and when ALS originates in this model. We provide evidence that the degenerative process in SOD1 rats begins in upper and lower motor neurons and not in the muscle, thereby supporting the “dying-forward” hypothesis of ALS.To further test this, we used AAV9-shRNA to knock down mutant SOD1 expression in the brain of SOD1G93A rats and have shown that this results in a significant delay of disease onset and extension of survival. Studies such as these will provide insight into the origin of degeneration of ALS in order to develop potential therapeutic approaches.

Efficient derivation and characterization of human induced pluripotent stem cells (hiPSCs) from fresh and immortalized blood cell lines at the iPSC Core Facility

Loren Ornelas, Robert Barrett, Anais Sahabian, Berhan Mandefro, Lindsay Lenaeus, Clive Svendsen, Dhruv Sareen

The Regenerative Medicine Institute iPSC Core facility specializes in the efficient generation of patient iPSC lines from various somatic tissues, such as blood (fresh and immortalized lines), skin fibroblasts, epithelial cells, and adipose cells. The Core has over 60 non-integrating iPSC lines from individuals with Spinal Muscular Atrophy, Huntington's disease, Amyotrophic Lateral Sclerosis, MCT8 deficiency, Charcot-Marie-Tooth Disease, Skeletal Dysplasia, Alzheimer's Disease, Diabetes Incontinentia Pigmenti and Neurofibromatosis 1. Each line has 3 or greater fully characterized, high quality clonal lines. In addition, stably modified nuclear GFP and luciferase expressing PSC lines have been generated. We have now also developed an efficient method for the generation of iPSCs from human lymphoblastoid B-cell lines (LCLs) and fresh whole blood. Patient iPSC lines from EBV LCLs and fresh blood were generated using the Amaxa transfection system under 21% O2. Parent cells were transfected with non-integrating oriP/EBNA1 plasmids that rely on episomal expression of OCT3/4, SOX2, KLF4, L-MYC, LIN28, SV40LT, p53 shRNA and EBNA1. EBV LCL cultures were then plated on Matrigel and fed a non-serum containing medium supplemented with small molecules for 18 days then transitioned to mTeSR1. Fresh blood cultures were plated on a MEF feeder layer and fed Primate ESC Medium. Colonies resembling PSC morphology were picked and plated on Matrigel coated plates or MEFs. iPSCs were successfully generated from EBV LCLs and fresh blood with a reprogramming efficiency of 0.03-0.06%. Twelve clones were selected for further expansion and 3 clones were successfully characterized using pluripotency assays such as AP staining, ICC, EB formation, Pluritest analysis and G-band karyotype. The Core can provide comprehensive support to the scientific community by creating relevant human disease models by generating and characterizing non-integrating iPSC lines from various somatic cells, including blood cell lines. We are now developing cutting-edge techniques involving iPSC technology, such as genome-edited isogenic and reporter PSC lines and dynamic suspension PSC cultures.

Targeting C9ORF72 with antisense oligonucleotides in C9-ALS iPSC derived motor neurons

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Amyotrophic lateral sclerosis (ALS) is a late onset neurodegenerative disease characterized by motor neuron loss in the brain and spinal cord followed by paralysis and death, within 3-5 years. The majority of cases are sporadic with ~10% being familial. Although the disease mechanism is not known, a recent discovery of a GGGGCC repeat expansion in the noncoding region of the C9ORF72 gene was found to be the most common cause of ALS (C9-ALS). To investigate the role of C9orf72 repeat in C9-ALS pathogenesis, we generated patient derived motor neurons from the fibroblasts of four C9orf72 hexanucleotide expansion carriers. Analyzing these motor neuron cultures (MNs), we detect no

significant differences in C9orf72 expression between C9-ALS MNs and control (CTR) MNs at the RNA/protein level, although we do see significant differences in transcriptional profiles in C9-ALS compared to CTR-MNs, using RNA-seq. We have confirmed several upregulated genes, including DPP6, which was previously implicated in ALS. Furthermore, we can reverse these transcriptional changes using antisense oligos (ASOs) to knockdown the C9orf72 transcript. Based on our current data, we suggest knocking down C9orf72 with ASOs may be a relevant therapeutic strategy for treatment of C9-ALS.

The non-ciliated luminal cells are facultative lung stem cells that are regulated by cell intrinsic and extrinsic changes.

Samriddha Ray

The epithelial lining of pulmonary airspaces is derived from developing anterior foregut endoderm through a process by which mesenchyme guides the fate of multipotent progenitors to yield lineage committed regional progenitors. We have employed in vivo genetic lineage tracing and cell fractionation approaches to define intrinsic and microenvironmental factors that influence the proliferative and differentiation potential of distinct regional epithelial progenitor cells of the postnatal lung. Evidences from transplantation and culture assays demonstrate that nonciliated luminal progenitors of mouse conducting airways are multipotent and can generate pseudostratified epithelium including all specialized cell types of the tracheobronchial airway. Additionally, we found that the in vitro regeneration capacity for multipotent differentiation is enriched by Stem cell antigen (Sca1) selection. Using physiologically relevant injury models such as those resulting from influenza virus we found that under conditions of severe airway injury luminal cells of both bronchial and bronchiolar airways demonstrate the capacity for generation of a pseudostratified epithelium indicative of their multipotency. These studies reveal unexpected multipotency of epithelial cells that maintain the lung epithelium and suggest that both stem cells and facultative stem cells function in close proximity to maintain the epithelium in normal and diseased states.

Safety and efficacy of repeat dosing of allogeneic cardiosphere-derived cell therapy after myocardial infarction in rats.

Heidi Reich, Eleni Tseliou, Konstantinos Malliaras, Geoffrey De Couto, Baiming Sun, Daniel Luthringer, Linda Marban, Eduardo Marban

When administered as a single dose, allogeneic cardiosphere-derived cells (CDCs) improve cardiac function, reduce scar, and increase viable myocardium in the infarcted rat heart without eliciting a detrimental immune response. Clinical trials using single doses of allogeneic human CDCs are underway. Whether a second dose of cell therapy offers additional clinical benefit, and/or results in sensitization, remains uninvestigated. Wistar Kyoto rats underwent surgical ligation of the left anterior descending

coronary artery and immediate intramyocardial injection of CDCs, followed by redo-thoracotomy and second CDC injection at three weeks. Treatment permutations included two doses of syngeneic Wistar Kyoto CDCs (syn/syn group, n=16; no sensitization expected), two doses of allogeneic Brown Norway CDCs (allo1/allo1 group, n = 16; maximal likelihood of sensitization), allogeneic Brown Norway then allogeneic Buffalo CDCs (allo1/allo2 group, n = 16), and dual saline injections (control group, n=8). Cardiac function was assessed via transthoracic echo at 24 hours, 3 weeks, and 6 weeks post-infarction, after which the hearts were harvested for histologic analysis. Repeat dosing of syngeneic and allogeneic CDCs improved left ventricular ejection fraction. Greater incremental functional benefit of the second dose of CDCs in the syngeneic group compared to the allogeneic group was suggested in this preliminary study; however, this trend was not associated with corresponding changes in infarct size or viable mass. The allogeneic and syngeneic groups both had reduced scar size relative to controls. Previous studies from the Marbán lab found that allogeneic CDC therapy was safe and promoted cardiac regeneration without need for concurrent immunosuppression. The preliminary results presented here suggest that there may be an incremental benefit of a repeat dosing of CDCs following acute myocardial infarction in the rat. Further study of the immune response to repeat dosing with allogeneic CDCs is under way.

Generation of Human Neural Progenitor Cells from Induced Pluripotent Stem Cells that Survive, Migrate and Integrate into the Rodent Spinal Cord

Anais Sahabian

Transplantationof human neural progenitors (hNPCs) derived from pluripotent stem cells (PSCs) is a promising therapeutic strategy that has the potential to replace lost cells, modulate the injury environment and create a permissive milieu for the protection or regeneration of host neurons in disease. Here we use a novel chopping technique to isolate and expand EZ spheres (Ebert et al., 2013) from PSCs, which were then driven to expandable iPSC-derived neural progenitor cells (iNPCs) with spinal cord phenotype using a combination of all-trans retinoic acid (ATRA) followed by the mitogens EGF and FGF-2. Induced NPCs grown in suspension showed similar characteristics to NPCs derived from human fetal tissues, although iNPCs grown in adherent cultures did not. Suspension iNPCs were easy to maintain using the chopping method of expansion and survived grafting into the spinal cord of athymic nude rats with no signs of overgrowth, again with very similar profiles to NPCs derived from human fetal tissues. These results suggest that iPSC-derived NPCs could be a favorable alternative to fetal NPCs for cellular regenerative therapies of CNS diseases. In the future, we will derive other CNS region specified iNPCs by patterning with specific cytokines, morphogens and small molecules.

Induced Pluripotent Stem Cells Derived from a Patient with Skeletal Dysplasia Display Abnormal Chondrogenic Markers Expression and Regulation by BMP2 and TGFbeta1

Biagio Saitta, Jenna Passarini, Dhruv Sareen, Loren Ornelas, Anais Sahabian, Deborah Krakow, Daniel Cohn, Clive Svendsen, David Rimoin

Skeletal Dysplasias (SDs) are caused by abnormal chondrogenesis during cartilage growth plate differentiation. To study early aberrant cartilage formation in vitro, we generated iPSCs from fibroblasts of an SD patient with a lethal form of metatropic dysplasia, caused by a dominant mutation in the calcium channel gene TRPV4. When mutant TRPV4-iPSCs were grown for chondrogenic differentiation and compared to control iPSC micromasses, decreased expression of growth plate cartilage markers: COL2A1 (IIA and IIB forms), SOX9, Aggrecan, COL10A1 and RUNX2, was observed by qPCR. We found that stimulation with BMP2, but not TGFbeta1, up-regulated COL2A1 (IIA and IIB) and SOX9 gene expression, only in control iPSCs. COL2A1 (Collagen II) expression was confirmed by Western blot and immunofluorescence microscopy. Alcian blue stain for proteoglycans was seen throughout the micromass sample in control, while TRPV4-iPSCs showed only focal areas of stain. Similar staining patterns were found in neonatal cartilage from control and patient samples. COL1A1 (Collagen I), an osteogenic marker, was upregulated at the mRNA level ($p>0.005$) in TRPV4-iPSCs compared to control, and confirmed at the protein level. Overall, the results validate that an iPSC model can recapitulate normal chondrogenesis and that mutant TRPV4-iPSCs reflect molecular evidence of aberrant cartilage developmental processes.

Therapeutic gene targeting to endogenous stem cells for bone regeneration

Dmitriy Sheyn, Gadi Pelled, Wafa Tawackoli, Susan Su, Zulma Gazit, Young Koh, Hyun Bae, Dan Gazit

Critical-size bone defects represent a great challenge for orthopedic surgeons. We have previously shown that MSCs engineered to overexpress BMP genes can differentiate and induce bone formation in vivo. To eliminate the need for MSC isolation, expansion and engineering, we sought to develop targeted BMP gene delivery to endogenous stem cells. We hypothesized that attraction of endogenous MSCs to a bone defect site, followed by ultrasound-based gene delivery would induce efficient bone regeneration. We first implanted a biodegradable scaffold in rat vertebral bone defects and mouse radius non-union fractures. After allowing MSC migration to the injury site for a few days, we injected a Luciferase or BMP plasmids into the bone defects followed by a short pulse of ultrasound. We found host endogenous MSCs migration to the site of scaffold implantation and subsequently were the target for the delivered transgene as characterized using immunostaining and flow cytometry. Luciferase was transiently expressed at the defect sites for two weeks as monitored non-invasively and longitudinally using BLI. Significant bone regeneration was detected with µCT in animals treated with targeted BMP gene delivery. Recently we were able to show the feasibility of this approach in a minipig model of segmental bone defect.

PTH treatment enhances endogenous mesenchymal stem cell differentiation, improving the integration of calvarial bone allografts

Wafa Tawackoli, Dmitriy Sheyn, Xiaoyu Da, Gadi Pelled, Dan Gazit, Zulma Gazit

Bone allografts have potential in craniofacial bone repair, although they often fail to integrate with the host bone due to scar formation. We hypothesized that intermittent administration of parathyroid hormone (PTH) would enhance MSC recruitment and differentiation, resulting in allograft osseointegration. Calvarial bone defects were created in transgenic mice, expressing Luciferase under osteocalcin promoter. Allografts were implanted in the defects, with or without daily PTH treatment. Endogenous MSC differentiation, as detected by bioluminescence imaging, was over 2-fold higher in mice treated with an allograft implant and PTH than in control mice. Fluorescent imaging demonstrated that the bone mineralization in PTH-treated allografts was significantly higher than that in untreated allografts. The mCT scans revealed a significant increase in bone formation in PTH-treated mice comparing to controls. The osteogenic genes Oc/Bglap and Ibsp were upregulated following PTH treatment. Interestingly we found a decrease in mast cells and Ang-2 expression with PTH treatment. In summary, PTH enhances endogenous MSC differentiation and bone formation around structural allografts. The precise mechanism is not clear, but we show that the infiltration pattern of mast cells, associated with the formation of fibrotic tissue, in the defect site is significantly affected by the PTH treatment.

iPSC-derived neural progenitor cells preserve vision in a rodent retinal degeneration model

Yuchun Tsai, Bin Lu, Sergey Girman, Anais Sahabian, Dhruv Sareen, Clive Svendsen, Shaomei Wang

Background: Stem cell therapy offers great potential for treating retinal degenerative diseases, and several clinical trials are now recruiting. Recent research has focused on replacing devastative cells or maintaining ocular environment trophically. Potential sources of stem cells for cellular therapy of primary retinal epithelium cell (RPE) defect should be able to clear debris from undigested photoreceptor outer segments (POS) and promote photoreceptor survival. Here we explored the potential of human induced pluripotent stem cell-derived neural progenitor cells (iNPCs) to rescue vision and possible mechanisms of action underlying the neuroprotective effect in the Royal College Surgeon (RCS) rat, a well-established model for age-related macular degeneration (AMD). Methodology/ Principal Findings: iNPC were transplanted into the subretinal space of RCS rats at 21 days postnatal (P21). Photoreceptors were preserved and visual functions were significantly preserved at P90-100 compared with controls using visual acuity measurements, electroretinography, and luminance threshold recordings from superior colliculus. The in vitro phagocytosis of POS by iNPC was examined by flow cytometry, immunocytochemistry, and western blot after feeding naive or FITC labeled POS. The evidence of in vitro phagocytosis was monitored by electro microscopy. Conclusions: The results underscore the potential therapeutic utility of iNPC and phagocytosing of POS as potential mechanism of functional rescue in retinal degeneration.

Is stop-flow necessary for efficacious intracoronary cell delivery? Comparison with nonocclusive intracoronary infusion in a porcine model

Eleni Tseliou, Hideaki Kanazawa, James Dawkins, Konstantinos Malliaras, Michelle Kreke, Rachel Smith, Supurna Chowdhury, Ryan Middleton, Daniel Luthringer, Linda Marban, Raj Makkar, Eduardo Marban

Background: Since the first intracoronary delivery of cells to the heart in 2001, the stop-flow technique has been used by default. A balloon angioplasty catheter is inflated in the target vessel and cells infused via the luminal port. However, the need for balloon occlusion has never been demonstrated.Objective: We sought to compare the safety and efficacy of intracoronary delivery of allogeneic cardiosphere derived cells (CDCs) under stop-flow versus nonocclusive intracoronary delivery in a porcine model of myocardial infarction (MI).Methods: MI was created by 2.5 hr occlusion of the mid-LAD in adult female Yucatan minipigs. Three weeks later, 12.5M CDCs were intracoronarily infused in the LAD under stop-flow (in 3 3-min occlusions with 3 min rest intervals) or nonocclusive conditions (continuous-flow infusion over 10 min; n=5 in each group). During the course of the study, animals underwent MR imaging at baseline (just before infusion) and 4 weeks post infusion. Left ventricular ejection fraction, scar mass and viable mass were evaluated at both time points.Results: No adverse events (arrhythmias, death) were observed during or soon after cell infusion in any of the animals infused. Coronary blood flow evaluated by TIMI grade was TIMI 3 in all animals following completion of infusion. TnI and CK-MB values were within normal range 1 day post-infusion in all animals. One month post-infusion, allogeneic CDCs reduced scar mass in both groups (continuous flow p=0.015 vs. baseline; stop-flow p=0.044). The effects on ejection fraction (p=0.08) and viable mass (p=0.88) were equivalent in the two groups.Conclusions: Nonocclusive continuous-flow delivery is equally efficient to stop-flow method as a means of cell delivery to the infarcted myocardium, at least for this particular cell type. The need for stop-flow delivery, while traditional, is therefore questionable.

Searching for abnormalities in AHDS - iPS cell derived oligodendrocytes to model psychomotor retardation in a dish

Gad Vatine

Mutations in the thyroid hormone (TH) transporter Monocarboxylate transporter 8 (MCT8) have been recently associated with the Allan-Herndon-Dudley Syndrome (AHDS). This rare genetic disorder is characterized by a combination of severe psychomotor retardation accompanied by abnormal serum TH levels. Although the endocrinological phenotype of MCT8-deficiency is well characterized, the mechanism underlying its neurological phenotype is still poorly understood. Delayed myelination has recently been reported in several AHDS patients and is becoming the most prominent pathophysiological symptom of AHDS.The immergence of induced pluripotent stem (iPS) cell technology has opened new avenues for modeling genetic neurological diseases. Here we reprogrammed fibroblasts derived from AHDS patients and controls into iPS cells. The gene expression profile and

neural differentiation potential were compared in patient and control iPS cells. To study the mechanisms underlying delayed myelination in AHDS patients, the iPS cells were differentiated into oligodendrocytes using established protocols. Interestingly, TH is an indispensable factor in the differentiation of oligodendrocyte progenitors into mature oligodendrocytes. Following differentiation, oligodendrocytes were cultured in various TH concentrations and assessed for survival, morphology and TH-responsive gene expression. Identifying abnormalities in cells derived from AHDS patients may serve as a screening platform for therapeutic agents for the treatment of MCT8-deficiency.

Reproductive Biology

Differentially Regulated Genes in Pregnancies Conceived in Couples with Infertility

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Infertility affects about 6.1 million people in the US, thus the use of in vitro fertilization (IVF) and other non-invasive fertility treatments such as controlled ovarian hyperstimulation (COH) and intrauterine insemination (IUI), has risen dramatically. These pregnancies are at increased risk of adverse outcomes, many of which may result from abnormal placentation and first trimester trophoblast function. Our goal is to determine whether these increased risks result from the fertility treatments used or from the underlying infertility. We used surplus tissue from chorionic villus sampling (CVS) obtained through the Prenatal Biorepository to examine first trimester trophoblasts from pregnancies conceived spontaneously vs. those conceived by infertile couples using IVF or COH/IUI. Microarray analysis revealed that there are a select group of genes that were up- or down-regulated in both infertile groups, and some that were specifically upregulated in the IVF group. As the TGF-β family plays a crucial role in regulating trophoblast cell proliferation, differentiation, adhesion, and invasion/migration, we first used siRNA to knock down expression of candidate genes related to TGF-β family functions. siRNA knockdown of these genes affected other genes related to TGF-β family functions, which may contribute to the adverse outcomes associated with pregnancies conceived using fertility treatments.

Reduced ovarian reserve is associated with BRCA1 germline mutations

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Objective: To determine if BRCA1 and BRCA2 germline mutations are associated with a decreased ovarian reserve, as measured by serum anti-Mullerian hormone (AMH) levels.Design: Cross-sectional studySetting: Academic cancer centerPatients: One hundred forty-three reproductive-age women who underwent clinical genetic testing for BRCA deleterious mutations due to a family history of cancer were classified into three groups: BRCA1 mutation carriers, BRCA2 mutation carriers, and non-mutation carriers. None of the women had a personal history of breast or ovarian cancer.Intervention: None Main Outcome Measure: difference in serum AMH level across the three groupsResults: BRCA1 mutation carriers had a 50% decrease in AMH levels compared to non-mutation carriers after adjusting for age and body mass index (BMI) (0.53 ng/mL 95% CI 0.33-0.77 vs. 1.05 ng/mL 95% CI 0.76-1.40). Logistic regression analysis confirmed that BRCA1 mutation carriers had a 4-fold higher odds of having AMH <1 ng/mL compared to non-mutation carriers (OR=4.22, P=0.012). There was no difference in AMH levels between BRCA2 mutation carriers and non-mutation carriers.Conclusions: BRCA1, but not BRCA2, mutation carriers have lower age- and BMI-adjusted serum anti-Mullerian hormone levels compared to non-mutation carriers.