ATHEROSCLEROSIS AND CARDIOVASCULAR DISEASE

ACD1 Development of the First Animal Model of Dyslipidemia, Atherosclerosis, and Diabetes in Mutant apoE Deficient/Zucker Diabetic Fatty (ZDF) Rats

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Cardiovascular disease caused 788,000 deaths in 2010. Atherosclerosis is a chronic disease influenced by increased lipids inside of injured arteries. Diabetic patients have aggressive and accelerated atherosclerosis. No small animal model appropriately resembles human diabetic atherosclerosis. We hypothesize that modifying lipid metabolism in Zucker Diabetic Fatty (ZDF) rats promotes dyslipidemia and atherosclerosis. We genetically modified lipid metabolism, by knocking out apolipoprotein E (apoE) gene using CRISPR/Cas9. The resulting animal model should be diabetic, obese, dyslipidemic, and atherosclerosis-prone. Using CRISPR/Cas9 the genome of ZDF rats was edited targeting apoE. Gene sequence and immunoblot of serum apoE was performed. Two different experimental cohorts were used. First cohort, CRISPR founders under normal diet at baseline and nine months of age. Second cohort, apoE-KO,ZDF 1st generation rats from selected CRISPR founders, were placed under high-fat diet with weight, lipids, blood chemistry, and glycemia assessment. Oral glucose tolerance test was used to diagnose diabetes. Rats were euthanized at 2-weeks, for aortic vascular lesions via Oil Red O (ORO) staining. Gene sequencing showed several apoE mutations at exons 1,3, or both. Immunoblot confirmed lack of apoE in serum. ApoE$^{-/-}$ rats develop severe hypercholesterolemia; apoE$^{-/-}$;Lepr $^{fa/fa}$ had ~2-fold increased cholesterol compared to apoE$^{-/-}$;Lepr$^{+/?}$. Females appear to be more severely dyslipidemic than males. 1st cohort apoE$^{-/-}$ rats developed increased aortic lipid deposit (thoracic,abdominal) compared to apoE$^{+/+}$ (P = 0.0002). 2nd cohort apoE$^{-/-}$ rat show increased aortic lipid deposit compared to apoE$^{+/+}$ (P = 0.0008). Aortic leaflets evidenced subendothelial lipid deposit in both cohorts only in apoE$^{-/-}$. Diabetes of the1st generation apoE$^{-/-}$;Lepr $^{fa/fa}$ males was confirmed by oral glucose tolerance test. We successfully generated apoE-KO,ZDF rats, the first polygenic rat model to study vasculopathies in diabetes. Future studies will evaluate our animal model longitudinally and further evaluate accelerated development of diabetes and atherosclerosis.

Supported by the NORC-PPF (P30 DK056350) grant to Dr. Edward Bahnson.

ACD2 Impaired Production and Diurnal Regulation of Vascular $RvD_{n\text{-}3\ DPA}$ Increases Systemic Inflammation and Cardiovascular Disease

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Diurnal mechanisms are central to regulating host responses. Recent studies uncovered a novel family of mediators termed as specialized pro-resolving mediators (SPM) that terminate inflammation without interfering with the immune response. Little is known on their diurnal regulation.

Herein we investigated the diurnal regulation of SPM in humans and their role in controlling peripheral blood leukocyte and platelet activation. Using lipid mediator profiling and healthy volunteers it was found that plasma concentrations of n-3 docosapentaenoic acid–derived D-series resolvins ($RvD_{n\text{-}3\ DPA}$) were regulated in a diurnal manner. The production and diurnal regulation of these mediators was markedly altered in patients at risk of myocardial infarct. These changes were associated with decreased 5-lipoxygenase expression and increased systemic adenosine concentrations. A significant negative correlation was also found between plasma $RvD_{n\text{-}3\ DPA}$ and markers of platelet, monocyte, and neutrophil activation including CD63 and CD11b. Incubation of $RvD_{n\text{-}3\ DPA}$ with peripheral blood from healthy volunteers and patients with cardiovascular disease significantly and dose-dependently decreased platelet and leukocyte activation. Furthermore, administration of $RvD5_{n\text{-}3\ DPA}$ to apolipoprotein E deficient mice significantly reduced platelet-leukocyte aggregates, vascular thromboxane B_2 concentrations and aortic lesions. These results demonstrate that peripheral blood $RvD_{n\text{-}3\ DPA}$ are diurnally regulated in humans and dysregulation in the production of these mediators may lead to cardiovascular disease.

ACD3 Wnt5a and Wnt3a Differentially Regulate Vascular Smooth Muscle Cell Proliferation and Migration

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Vascular pathology and cardiovascular disease (CVD) still are the first cause of death in modern societies. In the pathogenesis of atherosclerosis, migration and proliferation of vascular smooth muscle cells (VSMC) are important processes in plaque development. Proliferation and migration of VSMC from the media towards the intimal layer is associated with fibrous cap formation on top of the plaque, of which cap thickness is a key indicator of plaque vulnerability. The role of Wnt signaling in the pathogenesis of atherosclerosis is complex and is not well understood. We hypothesize that Wnt5a and Wnt3a are associated with plaque vulnerability and stability in the context of atherosclerosis.

Human carotid atherosclerotic tissue and human carotid VSMCs were used to investigate the role of Wnt5a and Wnt3a in atherosclerosis. Immunohistochemistry and Western blot was used to evaluate expression of Wnts in human tissue and VSMCs, respectively. Scratch assay and MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay were used to evaluate VSMC migration and proliferation. Expression of Wnt5a and Wnt3a were evaluated in VSMC in the intimal and medial layers of the artery as well as the fibrous cap. Scratch assay results indicate that Wnt5a promotes VSMC migration over 24 hours, whereas Wnt3a inhibits migration. Additionally, MTS proliferation assay results suggest that Wnt5a does not increase VSMC proliferation, whereas Wnt3a strongly increases proliferation to a similar degree as PDGF-BB (control). Interestingly, Wnt5a appears to induce quiescence in proliferating VSMCs. In conclusion, these results suggest that Wnt5a and Wnt3a play distinct roles dictating VSMC plasticity in atherosclerosis and manipulation of Wnt5a/Wnt3a expression may be a therapeutic target.

BREAST CANCER

BC1 Discoidin domain receptor tyrosine kinase 2 (DDR2)-depleted Mesenchymal Stem Cells Attenuate the Tumor-promoting Effect of Breast Cancer Cell Engulfment

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Mesenchymal stem cells (MSCs) are recruited to the tumor microenvironment and promote tumor progression. We have demonstrated that MSCs expressing discoidin domain receptor tyrosine kinase 2 (DDR2), mediate stromal-breast cancer interactions and promote metastasis. Pathologists have noticed cell "cannibalism" in breast metastasis. We hypothesize that breast cancer cells engulf MSC that enables metastatic dissemination, and that DDR2 inhibition in MSC may block this effect. We used GFP labeled breast cancer cells (BCC) metastatic (MDA-MB-231 and -436), non-metastatic (MCF7), and non-tumorigenic (HME and MCF10A), and Ds-RED labeled MSC controls (MSC-shC) and with *DDR2* shRNA knockdown (MSC-shDDR2). 3D-Co-culturing MSC-Ds-Red and BCC-GFP and flow cytometry were used to quantify cell engulfing. A microfluidic high-throughput cell paring and retrieval platform was developed to study BCC-MSC-shC and shDDR2 engulfing clones which allowed selective retrieval of single cells. RNA sequencing of engulfing MSC-shC or MSC-shDDR2 BCCs clones was compared to non-engulfing BCCs. The *in vitro* and *in vivo* relevance of engulfment of MSC-shC or MSC-shDDR2 by BCCs was assessed by WB, invasion, migration, mammosphere assays, and xenografts. Subpopulations of metastatic MDA-MB-231 and -436 cells engulf MSCs. MSC engulfment was not detected in non-metastatic and non-tumorigenic breast cells. Using our high-throughput cellular pairing platform MSC engulfment was visualized and engulfing and non-engulfing BCCs retrieved. RNA sequencing revealed a 7-gene engulfing signature by comparing BCC engulfing MSC-shC, and BCC engulfing MSC-shDDR2, BCC non-engulfing. Functionally, MSC engulfment enhances EMT, mammospheres, migration and invasion of BCCs. We developed a high-throughput cellular pairing platform to study MSC engulfment by BCC, and demonstrated that engulfment of MSC by BCC is a key mechanism enabling tumor progression. Our study suggests that *DDR2* knockdown in MSC reduces their ability to enhance tumorigenic functions after engulfment by BCC. We identify an MSC engulfment gene signature with potential for development of new tissue-based biomarkers of metastasis.

BC2 Star Related Lipid Transfer Protein 10 as a Novel Key Player in Ethanol-Induced ERBB2 Breast Cancer Progression

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Alcohol induces ErbB2 Receptor Tyrosine Kinase 2 (ErbB2) oncogene in breast cancer (BR). Star-related lipid transfer protein 10 (StarD10), a lipid transporter of phosphatidylcholine and phosphatidylethanolamine, essential for lipid metabolism and membrane fluidity, is highly expressed in 35% of ErbB2-positive BR. Our aim is to investigate the role of StarD10 and ErbB2 cross-talk in BR under ethanol administration and elucidate the molecular mechanisms. MCF-7 and SKBR-3 cell lines were used to analyze mRNA (Real-Time PCR), protein levels (Western Blotting), StarD10 promoter activity (reporter assay), cell proliferation (MTT) and phosphatidylcholine (enzyme-coupled). Ethanol-treated cells exhibited increased StarD10 and ErbB2 expression. Consistently, ErbB2 overexpression caused an increase of StarD10 expression. Overexpression of ErbB2 downstream (p65, c-MYC, c-FOS, c-JUN) induced StarD10 promoter activity mimicking ErbB2 trigger function. Also, we demonstrated that StarD10 and ErbB2 positively regulated each other's expression. Moreover, ethanol increased phosphatidylcholine level supporting the proposed role of StarD10 as player on tumorigenesis via its lipid transporter function. Interesting, both Stard10 overexpression and silencing induced cell growth and migration in MCF-7 and SKBR-3 cells. These data validated the hypothesis that StarD10 is a key protein in ethanol-induced breast cancer development accordingly with its pathophysiological level. High level of secreted PC was found in ethanol-treated cell media, however *StarD10* silencing completely prevented it. In contrast, *StarD10* overexpression promoted PC secretion and induced it further in co-treatment with ethanol. This finding may explain how StarD10 controls the changes in cell membrane properties during malignancy, which may be modulating the membrane fluidity. The ability of Stard10 to influence ErbB2 expression and activity may involve both dependent and independent lipid binding function. This is the first report demonstrating that ethanol can dynamically modulate the ErbB2 role through StarD10 involvement in breast cancer.

BC3 Characterization of the Role of L3MBTL3 in Breast Cancer

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Consistent with its role in mammary gland development and its broad contribution to oncogenesis across tissues, the Notch signaling pathway contributes to breast carcinogenesis (BC). We recently discovered and characterized a molecular interaction between RBPJ, a key transcription factor for the mediation of Notch signaling, and L3MBTL3, a methyllysine reader. We also demonstrated the role of L3MBTL3 in the RBPJ-dependent repression of Notch/RBPJ target genes in the triple-negative-BC cell line MDA-MB-231. The *L3MBTL3* gene locus is frequently deleted in BC and it contains several BC risk-associated single nucleotide polymorphisms (SNPs). Moreover, BC tumors have 39% less abundant *L3MBTL3* mRNA transcripts compared to normal tissue and high level of *L3MBTL3* expression is associated with longer relapse-free times. These independent studies indicate that *L3MBTL3* is a putative suppressor of BC. We hypothesize that *L3MBTL3* KO in the mammary gland may promote tumorigenesis through aberrant "de-repression" of Notch/RBPJ target genes. In support of this hypothesis, recent studies in our laboratories showed that *L3MBTL3* KO in MDA-MB-231 cells leads to a distinct phenotype characterized by higher metabolic activity and an increase in aldehyde dehydrogenase activity. Moreover, *L3MBTL3* KO MDA-MB-231 cells show increased propensity to form tumorspheres, suggesting that *L3MBTL3* regulates cancer stem cells. To investigate *in vivo* the physiological consequences