

Voelcker Biomedical Research Academy

Class of Summer 2013

Graduating Scholar Abstracts

Bright Streaks as an Indicator of Thin-Cap Fibroatheroma in OCT Images

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Introduction

Heart attacks are the most common cause of death in many developed countries. Optical coherence tomography (OCT), an invasive imaging modality used in the diagnoses of coronary arterial disease provides images whose interpretations are highly subjective and complicated, yet still helpful in the assessment of a patient's coronary health. Of the common coronary artery health issues present in patients is thin-cap fibroatheroma (TCFA), a type of vulnerable plaque with the potential to rupture and cause a myocardial infarction. My project utilizes the presence of bright streaks in coronary OCT images to verify the presence of a particularly dangerous TCFA, called a necrotic core. We hypothesize that the bright spots algorithm developed by our group will accentuate bright streaks for improved and quantitative identification of TCFA.

Methods

The frames of OCT pullbacks from *ex vivo* human hearts in patients who had coronary atherosclerosis were evaluated for the presence of TCFA with and without the use of the bright spots algorithm. Histologically confirmed true thin-capped fibroatheroma were then matched to the regions of interest on the OCT pullbacks. The presence of bright streaks with and without the bright spots algorithm in the frames of the pullbacks were compared to the true and false thin-capped fibroatheroma as confirmed by the histology.

Results

The presence of the bright streaks in the frames of the pullbacks were compared to the true and false thin-capped fibroatheroma as confirmed by histology. Approximately 53% of the true TCFAs expressed the bright streaks before the algorithm, and increased to approximately 87%, indicating the algorithm detected a bright streak where one was not present before. A similar effect was also observed with the false TCFAs. The percentage in which bright streaks were observed decreased from 15% to 10% after application of the algorithm. This information was proven statistically significant according to a Students' paired t test, where there is sufficient evidence to suggest that after the algorithm is applied, the number of true TCFAs displaying bright streaks increases, and the number of false TCFAs displaying bright streaks decreases. The overall percentage of agreement between the presence of bright streaks and histology before the algorithm is approximately 70% which increased to approximately 80% after the application of the algorithm. Indicating that that the bright streaks are specific to TCFA and rarely are they seen in other types of plaque and deposits in the arterial walls, (including thick-capped fibroatheroma, and necrotic and lipid cores).

Conclusions

The hypothesis was partially supported because although the majority of the TCFA expressed bright streaks, there were several false TCFA also expressing a similar effect. The use of the bright streak algorithm as an additional method of verification of the presence of a thin-cap fibroatheroma and the use of the algorithm used in this project has the potential to more accurately diagnose patients at risk for future heart attacks. Histological confirmation is impossible for *in vivo* patients, and given the highly qualitative interpretation of OCT images, the identification of bright streaks in a quantitative fashion serves as a valid method for identifying TCFA with the subset of necrotic cores.

A comparison of thapsigargin-induced eIF2a phosphorylation in cells expressing PSP-associated PERK variants

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PERK, a stress-responsive eIF2a kinase in the endoplasmic reticulum (ER), is abnormally active in the brains of patients with Alzheimer's disease (AD) and Progressive Supranuclear Palsy (PSP). The inheritance of 3 non-synonymous coding variants (Ser136Cys, Arg166Gln, and Ser704Ala) in PERK is associated with PSP, suggesting that PERK influences PSP pathogenesis. Therefore, we investigated the specific effect that homologous coding variants (Ser132Cys, Arg162Gln, and Ser700Ala) in mouse PERK have on PERK activity by measuring the phosphorylation of PERK's substrate, eIF2a. In this study, we first found that each variant, as well as wild-type PERK, is capable of phosphorylating eIF2a when over-expressed in PERK-deficient mouse embryonic fibroblasts (MEFs). We also found that expression of Ser132Cys PERK increased the phosphorylation of eIF2a compared to wild-type PERK in response to the thapsigargin-induced ER stress. Our results show that these polymorphisms do not individually eliminate PERK activity, and that Ser132Cys enhances the activity of mouse PERK. This suggests that the homologous Ser136Cys coding change in human PERK may similarly affect its activity. Further work is required to more fully understand the effect that Arg162Gln and Ser700Ala may have on mouse PERK activity. Our data suggest that Ser132Cys alters the sensitivity of mouse PERK to ER stress, and possibly contributes to the elevated PERK activity in the brains of PSP patients. Moreover, as S132C resides in the ER luminal stress-sensing domain of PERK, these results highlight the importance of ER homeostasis in AD and PSP.

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APPL1-sf Negatively Correlates Insulin Sensitivity

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Introduction: Type 2 diabetes is a prevalent disease in which the mechanisms underlying insulin resistance development are not yet clearly understood. The hormone, adiponectin, binds to adiponectin receptors on the cell membrane and promotes insulin sensitivity in the body. One adiponectin receptor binding protein, APPL1, positively effects insulin sensitivity in cells and in animals. Currently, we have discovered an isoform of APPL1, APPL1-sf that is highly expressed in liver and pancreas tissues. Our cellular studies demonstrate that APPL1-sf inhibits adiponectin signaling. However, the correlation of this protein expression with insulin sensitivity is unknown.

Methods: Two animal models were used in this study. In the first study, wild type mice were calorie restricted with a reduction of 40% less calories than ad libitum mice for four months and showed enhanced insulin sensitivity. This second mouse model was db/db mice in which the leptin receptor gene was knocked out and heterozygous mice were the control. The db/db mice showed symptoms of diabetes including hyperglycemia, hyperinsulinemia and a significant increase in size due to increased fat storage, especially in the liver compared with the heterozygous mice.

Conclusion: APPL1-sf expression in db/db mice was significantly increased compared to in their controls. Reversely, caloric restricted mice had a decrease in APPL1-sf expression. APPL1-sf protein levels in liver tissues are negatively correlated with insulin sensitivity. This study suggests the APPL1-sf could be a biomarker for insulin resistance, a causal factor of type 2 diabetes.

TMEPAI (Transmembrane Prostate Androgen Induced), a Potential Circulating Biomarker for Triple Negative Breast Cancers

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Triple negative breast cancer (TNBC) is an extremely aggressive malignancy associated with high rates of morbidity and mortality. Identification of novel biomarkers that are secreted by this cancer cell type could help in early detection and monitor treatment response. Exosomes are 30- to 100-nm size micro-vesicles secreted by a wide range of mammalian cell types by exocytosis. However, abnormal release of exosomes occurs in many diseases including cancer. The magnitude of exosome release is often linked to tumor invasiveness both *in vitro* and *in vivo*. Exosomes are small enough to penetrate into and interact with cells and tissues and have been shown to promote tumor cell proliferation, angiogenesis, escape from immune surveillance, extracellular matrix degradation, and create metastatic-niche at tissue sites, creating positive tumor growth environments for carcinogenesis. Previous research identified that exosomes assist in the spread of tumor growth as well as contribute to the therapy resistance, therefore making exosomes a possible therapeutic target. TMEPAI, a novel gene (transmembrane prostate androgen induced), is highly induced by TGF- β in cancer cells but not in normal cells resulting in the alteration of growth suppressive property of TGF- β into abnormal growth promotion. The objective of our research has been to detect TMEPAI in exosomes isolated from triple negative breast cancer cells. We isolated exosomes from MDA-MB-231 breast cancer cells treated with or without TGF- β using differential centrifugation techniques. Isolated exosomes were analyzed for the presence of TMEPAI as well as various tetraspanin markers (CD9, CD63 and CD81) and heat shock proteins. As a negative control we included exosomes isolated from TMEPAI knockdown cells (TMKD). Based on our results, we conclude that triple negative breast cancer cells MDA-MB-231 expressed and secreted TMEPAI through exosomes into the extracellular medium. This further justifies the hypothesis that TMEPAI containing exosomes may alter the tumor microenvironment by modifying TGF-beta signaling in the tumor milieu in such a way to create metastatic-niche and avoid immune surveillance.

Divergence Between Epithelial Cell Integrity and Inflammation in Allergic Rhinoconjunctivitis

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Background: Identifying the location on the gene that is responsible for the genetic expression that produces antibodies like Immunoglobulin E and others that cause histamine release at the introduction of an allergen to the body is key to permanently eliminating allergies in individuals. This is possible by comparing genetic expression in those who have immune responses to allergens to those who are unaffected by allergens. Investigating the genetic deviation in these individuals is the fundamental component in permanently eliminating allergies in individuals as opposed to merely alleviating symptoms.

Methods: RNA strands taken from epithelial cells of thirty-eight individuals, twenty-three with a history of allergies (categorized as HDM+) and fifteen with no history (categorized as HDM-), exposed to House Dust Mite (HDM) powder for one hundred and eighty minutes at fifty percent humidity and 22 degrees Celsius were prepared and sequenced, and this sequenced data was provided to the researcher. The data was then input into the program “R” using the “DESeq” package and a gene-expression matrix designating rows as genes and columns as gene counts in each sample was created. Two types of analyses were conducted, one cross sectional measuring the difference between positive and negative responders at specific time points (prior to exposure and every thirty minutes during the one hundred and eighty minute exposure), and a longitudinal comparison showing which genes showed increased expression throughout the full length of the study.

Results: The results of this study proved the hypothesis, as there were clear differences in expression of distinct genes between those who had a history of symptoms to allergens and those who expressed no symptoms. In those individuals who expressed symptoms to allergies, it was seen that genes related to immune response pathways and biological functions like chemotaxis, chemokine reception, and interleukin signaling were expressed at higher rates compared to those individuals who expressed no symptomology. In relation to these findings, it was also discovered that in those who expressed no symptomology, epithelial barrier genes were expressed much more than immune response genes indicating that stimulation of epithelial barrier genes combined with suppression of immune response genes will lead to reduction or elimination of reactions to allergens.

Identifying Effective Strategies for Obtaining Followers on a Health Issues Instagram Account

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Introduction: Substance abuse among teens is an important issue. With a startling number of teens using illicit drugs, 51.5% of 8th- 12th graders in 2012, we need to do something to stop this trend. The Opal Pain and Addiction Lab, OPAL, is interested in utilizing widely available social media platforms to deliver addiction-related public health interventions. Social media is now a major platform for communication, especially among teenagers. For example, 52% of all 13 to 17 year olds use Instagram on a daily basis, and health related messages can be spread fast through it. The challenge is identifying how to make a health issue, like teenage substance use, go 'viral.' Kim Kardashian has 30 million followers on her Instagram account, but the few health related accounts we encountered only had 0-10 followers.

Research Objective: To learn more about strategies for using social media, the purpose of this project was to identify the best strategies to obtain followers in order to deliver health related messages to the youth population.

Approach: We created an Instagram account whose purpose was to deliver health issue messages to teenagers. Our primary goal was to get as many followers as possible in 3 weeks. The account started with 0 followers, and without any advertising, we were able to obtain a total of 6 followers within one week. Our strategy to create a presence on social media was to post at least three times a day using popular hashtags related to our posts. Although this strategy helped us get likes on our posts, it did not considerably increase our followers. I made a presentation to 1st year students that are part of the Voelcker Biomedical Research Academy and encouraged them to follow us. Later, I sent an email to the Voelcker directors asking them to encourage every student to follow our account, not only first years. We then used Instamacro, a website in which one pays to promote an Instagram account to other users who share similar interests based on their posts. After Instamacro, we started using drug related hashtags to focus our message to substance abuse.

Results: Before paying, Instamacro lets you test its strategy for three hours. During those three hours our followers increased from 6 to 23. Within a day, we lost a follower, and by the third day we were at 20 followers, and maintained there. We posted on average 3 to 5 posts per day. Our next step was to try a paid subscription to Instamacro; we tried it for one day to see how it worked. The likes and followers started accumulating, and by the next day we went from 20 to 77 followers. Similar to before, we started to lose followers and within one day we lost 3. Since the results after one day provided us with 74 followers, we tried Instamacro again, but this time for 2 days. The results were not as good as expected. We expected to get at least 750 followers, as Instamacro had predicted, but we reached a total of 115 followers after advertising for 3 days on Instamacro

Conclusions: Accruing Instagram followers to deliver health related issues is challenging. For 3 days of advertising, Instamacro alerted us stating we could expect up to 750 followers. We reached 115; still greater than most health oriented Instagram accounts. We expected the Instamacro advertising strategy to work the best in terms of gaining followers, and posting 3-5 times a day to be least effective. Instamacro did work the best, helping us get over 100 followers and 58 likes on our most liked post. Posting 3-5 pictures a day was not going to work as much if we did not have followers to see or like those posts. This suggests that potential followers elected not to follow us, maybe because our posts were not as appealing as we thought. We also expected to lose more than half of the followers after they realized we do not follow them back -the majority is looking for a follow back to get more followers, that is one of the reasons why they follow- but surprisingly, we only lost 5 followers. Raising awareness of health related issues is harder than expected. After this study, we hypothesize that if we make a more direct call to action, the amount of followers and interested people will increase. Something like "today is the day," "visit our website now," or "get help now" can be really helpful to cause an immediate response from the audience. Science is an iterative process; we have to try over and over again with different strategies to see which strategy is more successful. By doing this, we should get better results over time. A more direct call can provide us with a communication vehicle to reach teenagers and provide them with helpful information using social media.

Assessing the antidepressant-like efficacy of ketamine in KMO-deficient mice

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Ketamine is a drug that is commonly known for being used as an anesthetic and as a possible antidepressant as shown in previous research. When a low dose of ketamine is administered it can give an anti-depressant treatment but when ketamine is administered in a larger dosage then it can result in sedative effects. In past research with ketamine, we have observed that in mice lacking the kynurenine 3-monooxygenase (KMO) gene, the anesthetic effects of ketamine are blocked. The metabolism of tryptophan occurs through the kynurenine pathway, which then branches out into a neurotoxic, and a neuroprotective pathway. The enzyme that begins the neurotoxic pathway, Indoleamine 2,3-dioxygenase (IDO), is upregulated by constant immune activation that can occur in people with chronic illness. KMO is a key enzyme that is present after IDO on the neurotoxic side of the kynurenine pathway that then creates quinolinic acid that is an agonist for the N-methyl-D-aspartate (NMDA) receptor. We hypothesized that depressive-like behavior in the KMO knockout (KMO^{-/-}) mice treated with ketamine will be similar to KMO^{-/-} mice treated with saline. To determine the antidepressant-like effects of ketamine in KMO^{-/-} mice, we injected 10mg/kg ketamine or saline and assessed behavior 30 minutes, 24 hours and 7 days later. The behavioral tests that were conducted were the open field test (OFT) and the forced swim test (FST), which measure depression-like behaviors. First, the OFT was conducted over a 5 minute time period that assessed behaviors including the time spent in the central area of the box, distance traveled, and thigmotaxis or the tendency to remain around perimeter of the box. The FST was assessed on how much time the mice spent climbing, swimming, and floating over the 6 minute test period. Data from the OFT demonstrated that ketamine had no significant effect on locomotor activity. In the Forced Swim Test, 30 minutes after injection, focusing on the floating or immobility behaviors, the data show that all mice had a small amount of time spent floating in the beginning but then eventually increased their time over the 6-minute period. The FST data also show that the mice administered ketamine spent a decreased amount of time spent floating compared to the mice that were given saline. Similar results were seen 24 hours and 7 days following treatment with ketamine. This suggests that ketamine does have an antidepressant-like effect in KMO^{-/-} mice when we hypothesized that it would have no effect. So although a high dose of ketamine does not have an anesthetic effect in KMO^{-/-} mice, a low dose of ketamine does appear to have antidepressant-like effects in these same mice. These results may then cause further questioning for more research on the specific mechanisms that allow ketamine to have these effects in the brain.

Cx50 Second Extracellular Loop Domain in Cell-Cell Adhesion

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Background: Connexins are proteins that form gap junctions and hemichannels, which mediate communication between cells and cells to their environment, respectively. In lens, there are three connexins, Cx43, Cx50, and Cx46 and the function of these connexins are important for maintaining lens transparency and lens development. For example, Cx50 mutation causes microphthalmia and cataracts. Connexin molecules consist of nine domains, an N-terminus, four trans-membrane domains, two extracellular loop domains, an intercellular loop, and a C-terminus. Recent studies conducted in Dr. Jiang's lab have shown that Cx50 has adhesion function in the lens and the second extracellular loop domain (E2) is likely to be involved.

Purpose: 1) To determine Cx50 E2 domain function in cell-cell adhesion. 2) To identify critical amino acid residues in Cx50 E2 domain involved in cell adhesion function.

Hypothesis: Cx50 E2 domain mediates cell-cell adhesion in the lens and mutation of critical amino acid residue(s) in E2 domain will impair this function.

Methods: Amino acid sequences of E2 domains from various animal species found on the NCBI database were compared using BLAST and conserved amino acid residues were identified. The conserved amino acid residues, C185, C190, C196 and R200A were mutated to A using site-directed mutagenesis. Four DNA primers were designed and synthesized and polymerase chain reaction (PCR) assay was performed using Turbo enzyme and cCx50E2 in pGEX2T plasmid as a template using the Veriti® Thermal Cycler. The mutated plasmids were then transformed into an E. coli strain called DH α 5. Plasmid DNAs were isolated using Miniprep kit and DNA concentrations were determined using the Nanodrop device. The DNA was sequenced in the UTHSCSA DNA facility to ensure the correctness. The E. coli colonies containing mutated E2 domains were used to express GST-cCx50E2 by IPTG induction.

Results: Twelve potential amino acids residues were identified as candidates involved in cell adhesion function of Cx50 E2 domain. We successfully mutated the critical amino acid residues, C185A, C190A, and C196A. The fusion proteins were expressed in E. coli by IPTG induction and pure fusion protein will be isolated. These proteins will be used to test their roles in mediating cell-cell adhesion function using "parachuting" cell attachment and protein pull-down assays. These studies will provide fundamental understanding of the roles of connexin in physiology and pathology condition.

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Acute and Chronic Effects of Uptake 2 Blockade on Mouse Social Behavior

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Impaired social behavior is a core symptom of autism that also occurs in psychiatric disorders such as schizophrenia that is treatment-resistant. Better pharmaceutical interventions are greatly needed to treat these disorders. The goal of these studies was to determine if uptake 2 blockade can enhance social interaction in two mouse models of impaired sociability: black and tan brachyury (BTBR) and serotonin transporter knock-out mice. The uptake 2 transporter blocker decynium-22 (D-22) was administered to the mice at 0.001-0.01 mg/kg/day acutely by IP injection or chronically by mini osmotic pumps for 12 days. With the drug on board, the mice were subjected to three-chamber sociability tests. In these tests mice are pre-conditioned for a total of twenty minutes, and then tested for 10 min to determine preference for social interaction, and ten minutes for preference for social novelty. Two rounds of these studies were performed over two years such that 8-10 mice per treatment group were tested. The key finding was that in both SERT $-/-$ and BTBR mice, social behavior as measured by time in chambers or social sniff improved with acute or chronic D-22 treatment at doses of 0.01 - 0.1 mg/kg. This improvement occurred without loss of preference for social novelty. Following the threechamber test, the mice are put into an arena of fifteen marbles to test their social withdrawal. At the end of this test, we count how many marbles each mouse has buried and discuss the possible reasons for these results. Autoradiography was performed to determine if chronic subcutaneous D-22 treatment down-regulates uptake 2 binding sites in the brain, but those results are not yet available. If social behavior deficits are present they may stem from insufficient serotonergic neurotransmission, therefore we section off the frontal cortex and hippocampus using a cryostat to easily observe these parts of the brain. Our findings so far indicate that uptake 2 blockade may be an effective treatment for impaired social behavior, which warrants further study.

Verification of Bead Modeling System by Determining the Frictional Ratio and Partial Specific Volume of Various Proteins

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Understanding the exact structure of a given protein is essential for most experiments involving proteins. This information gives scientists critical insight about the function and how other substances interact with proteins. Previous modeling methods include x-ray crystallography, NMR spectroscopy, and electron microscopy; however, a new modeling method would allow a unique and possibly more precise results. This project focuses on verify a bead modeling method developed at IRCCS University hospital San Martino ist. In order verify their results, we acquired 5 well studied proteins (cytochrome c, carbonic anhydrase, lysozyme, chymotrypsinogen A , ribonuclease A) from Sigma-Aldrich. In order to ensure the purity of the proteins we dialyzed the proteins for two days in a buffer consisting of 5 mM Sodium Phosphate(pH 7). This process was designed in order to remove any DNA or RNA fragments, which would alter the results of this experiment. The proteins were then diluted into multiple precise concentrations along with varying concentrations of sodium chloride. We decided to use 7 different salt concentrations to investigate any effects that salt has on the structure of a protein. The dilutions were then run in a spectrophotometer to measure the absorbance (measured in OD). It is crucial to identify protein concentrations that results in an absorbance of .3 and .9 OD because the analytical ultracentrifuges used detect signal best at this absorption. Additionally, spectrophotometer data was used to determine what wavelength the proteins in the buffer absorb most(230 nm). The correct protein dilutions were then loaded and run in the analytical ultracentrifuge at 45000 rpm for approximately sixteen hours. We then analyzed the data using two main methods, 2DSA and PCSA. Both processes create a fit for the data while removing the time and radially invariant noise. Through these fits, we determined the frictional ratio and partial specific volume(\bar{v}) for each protein in each salt concentration. These two pieces of information will then be used to verify the information received from the proposed bead modeling system.

Developing Novel Composites for Use in Dental Caries

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I worked in the Biomaterials Research Group in the School of Dentistry, and focused on developing novel composites for use in dental caries, or cavities. Modern dental resins, made from Bis-GMA (bisphenol A glycidyl methacrylate) or TEGDMA (triethylene glycol dimethacrylate), last for approximately 10 years. My lab seeks to extend the life of dental resins through the formulation of polymers composed of an oxirane-acrylate system (OASys). The oxirane we are using is Epalloy (epoxidized, hydrogenated, bisphenol A), and the acrylate is DPHA, or dipentaerythritol penta/hexa acrylate. Combining the two results in an interpenetrating polymer network (IPN). This was hypothesized to result in higher durability, even more so when a silanated filler (such as the barium-glass acrylate filler we used) is added. To begin the curing process, which hardens the resin in molds, various chemical coreactors were used such as polytetrahydrofuran (THF250), octyloxy phenyl phenyl iodonium hexafluoroantimonate (OPPI), dimethyl aminoethyl methacrylate (DMEMA), and camphorquinone (CQ). We used a blue-light curing lamp to cure the samples, which become hardened disks known as buttons. We formulated buttons with varying proportions of oxirane to acrylate: 75% DPHA/25% Epalloy, 50/50, 25/75, and 100% DPHA. Three various initiator amounts were optimized. These, along with the control buttons formulated with Bis-GMA and TEGDMA, were tested for hardness, color, and hydrophobicity. We found that resins formulated with 70% filler were significantly harder than controls (Bis-GMA/TEGDMA). More data and results are pending.

We also developed an OASys-based bonding resin—the material used to secure the filling to the tooth wall— with silver nanoparticles (silver benzoate, AgBz) designed to release into the surrounding space. The antimicrobial properties of silver make it an ideal addition to the bonding agent. The addition of AgBz increased the degree of cure, and the color was controlled with low levels of bismuth oxychloride (BOC). Buttons with 0.025% AgBz, 25/75 and 50/50 Epalloy/DPHA, as well as buttons with 0.05% AgBz, 25/75 and 50/50 Epalloy/DPHA showed high levels of hardness. More data and results are pending.

Maternal Adiposity Dependent Alterations to Placental BDNF Expression and TRKB Phosphorylation

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The prevalence of obesity (BMI ≥ 30) in American women of childbearing age is still increasing. Epidemiological studies have found positive correlations between obesity and placental dysfunction. Brain derived neurotrophic factor (BDNF) is necessary for placental development and fetal growth. The action of BDNF is facilitated through tropomyosin related kinase B (TRKB), a receptor kinase that induces several signaling cascades. BDNF levels are lower in plasma of obese adults however the effect of adiposity on placental BDNF is not known. We hypothesized that placentas from obese women (OB; pre-pregnancy BMI >30) will have decreased BDNF expression and attenuation of TRKB autophosphorylation when compared to placentas from lean women (LN; BMI 18.5-24.9). Placental villous tissues were collected, in a randomized manner, following delivery by C-section at term with no labor, from LN or OB women with either male or female fetuses (n= 10 each group). BDNF mRNA expression, mature BDNF protein expression (inactive monomer and active homodimer), total TRKB protein expression, and TRKB phosphorylated at tyrosine 515 and 817 were quantified in total RNA and placental homogenates by RT-qPCR and Western blotting. There were no differences in BDNF mRNA across groups or in inactive BDNF monomer in placentas from LN compared to OB women. However, monomer expression was significantly greater in male when compared to female placentas of OB women ($p=0.005$). Conversely, active homodimer protein was significantly decreased in male compared to female placentas of LN women ($p=0.02$). Total TRKB protein expression was significantly decreased in female placentas from OB compared to LN women ($p=0.003$). TRKB autophosphorylation at tyrosine 515 was also significantly decreased in female placentas from OB compared to LN women ($p=0.01$). There were no differences in TRKB autophosphorylation at tyrosine 817 across groups. Our data show that BDNF expression is regulated at the translational level. Additionally, BDNF dimerization is altered in placentas of obese women with a male fetus (inactive BDNF) while less total TRKB and TRKB phosphorylated at tyrosine 515 is seen in obese women with a female fetus. This implies that maternal adiposity affects the placental BDNF/TRKB autocrine loop in a sexually dimorphic manner.

Maternal Immune Activation in Pregnant Mice Causes Abnormalities in Behavior

Kristian Rivas, Karen Jimenez, Jason O'Connor PhD

Autism is a neurodevelopmental disorder characterized by impaired social interaction, repetitive behavior and cognitive inflexibility. Autism is thought to result from the interaction between a susceptibility genotype and environmental risk factors. The offspring of women who are challenged by an infection during their pregnancy generally are at an increased risk for abnormalities in behavior such as autism. We hypothesize that the reason for this increased risk is because when the mother is challenged with an infection (in this case Poly I:C) it activates pro-inflammatory cytokines, which cause the up-regulation of IDO (an enzyme that in humans is encoded by the IDO1 gene) and begins to produce a neurotoxin called quinolinic acid. Exposure to this inflammatory prenatal environment may interfere with normal development. To begin testing on mice that would display this outcome, we set up the following behavior tests; marble burying and grooming. These tests help show typical autistic behaviors of disliking change in their environment and repetitive behavior. For the marble burying test 20 marbles were placed in a cage with a single mouse. The mouse then proceeds to "bury" the marbles. Mice that bury more marbles compared to the control mice are presumed to express autistic behavior referred to as 'insistence on sameness". For the grooming test we looked at the amount of time an individual mouse would spend grooming themselves. This shows repetitive behavior, which implies that the mouse is displaying autistic behavior. The mice were in four separate groups for the behavior tests; wild type (WT) with saline given to their mother during pregnancy (both controls), WT whose mothers receive Poly I:C during their pregnancy (control mice with immune challenge), IDO knockout mice whose mothers received saline (IDO making their offspring less likely to express autistic behaviors), and IDO knockout mice, whose mothers received the Poly I:C. The results of the behavior tests showed expected and unexpected behaviors. For the grooming behavior test we could see that it was the females that spent more time grooming themselves than their male counterparts. This was interesting considering that it was the males who were expected to show more of this behavior, since in humans, the prevalence of autism is significantly higher in males. For the marble burying, the results were what were expected. The offspring, whose mother were challenged by Poly I:C during pregnancy, had buried the most marbles compared to the control mice. From these results we can conclude that knocking out the IDO gene and little to no effect on the offspring's development of autistic behaviors.

Delivering Health Information and Identifying Strategies to Obtain Followers on a Twitter Account

Rodriguez, C.I., Guzman, D.G., Virk, H.S., & Potter J.S.

Background : Social media is a fast growing online resource used by nearly all adolescents. Social media is an outlet for information on local, national, and international affairs. Health communication specialists increasingly recognize the potential of social media to deliver health interventions. However, there is little health information on social media utilized by teens and young adults. Twitter, a social media platform, stated 33 percent of all 13 - 17 year olds (Generation X'ers) who use social media are active users. With these numbers there is a definite chance that health information can be shared with both groups. The challenge is to identify strategies to reach and engage these users with health information that might change substance use behaviors.

Purpose: The purpose of the study was to deliver health information (substance abuse, alcoholism, addiction etc.) to adolescents and millennials who are active users on Twitter and to see how many "followers" we could obtain in 1 month using our PeOPALstrong account. The overarching goal is to learn more about engaging this audience in health interventions that utilize widely used social media.

Approach: To start, we researched methods to obtain followers on Twitter. We started off by using common hash tags (#Summer, #Hope, #Love) then proceeding to more specific hash tags to narrow the target audience (#Drugs, #Teens, #Addiction, #SubstanceAbuse). According to research, posting at specific times will increase the chance of tweets being viewed; in response to this we tweeted 5 times a day during these times: 10 AM, between 12 PM- 1 PM, and 3 PM- 6PM. These times represent when most people would have downtime during their day and the most common times users would be active on Twitter. Timing of tweets isn't the only way to have a successful campaign, the content is also extremely vital. Tweeting articles and pictures was a great tool that provided a visual to attract users to engage in our tweets. Also we engaged in conversations with other Twitter users which would increase the chance of getting followers. After 2 weeks into the study, we used Twitter Ads to promote our account and reach out to a more selective audience (Adolescents, Millennials, Parents of Adolescents, etc.). We chose to launch 5 paid advertising campaigns (\$20.00 each) over a 15-day period that involved 4 cities (San Antonio, Houston, Dallas, Austin) and the entire state of Texas. The city campaigns had specific audiences and were scheduled for 2 days starting at 12 PM and ending at 11:59 PM the next day. The Texas campaign (\$60.00) lasted 4 days with the same start and end times with no specific audience. Additionally, we used Twitter Analytics to track our campaigns. It measured tweet engagements, tweet impressions, and followers we obtained from each campaign. Also Twitter Analytics tracked what kind of users followed our account, such as their demographics, interests, and hobbies.

Results: After the campaigns expired and data was collected, it showed we obtained 44 followers within the 1-month period. The following listed campaigns were used through Twitter Ads and obtained the amount of followers: San Antonio Campaign had 3 followers, Houston Campaign had 5 followers, Dallas Campaign had 5 followers, Austin Campaign had 0 followers, and the Texas Campaign had 2 followers. Out of the total 15 followers, only 2 were Generation X'ers and 1 was a Millennial. Before Twitter Ads, we had 29 followers 6 of them were Generation X'ers and 3 were Millennials. These followers were obtained by using common and specific hash tags or joining conversations on Twitter. A total count of 22.3K impressions (times a user is served a tweet in timeline or search results) were tracked during all of the campaigns.

Conclusions: After looking at the results, it has been concluded that being engaged with other Twitter accounts and using common and specific hash tags was a better strategy to gain followers. Twitter Ads was a great tool to use to get our PeOPAL account recognition but it did not engage with other users. Twitter Ads only helped by increasing the amount of impressions. However, these impressions were important in delivering health information to active users. This means that even though we did not get the high amount of followers we wanted, approximately 13.4K impressions was the combined total for Generation X'ers and Millennials which that there was a chance that an active user who is under substance abuse viewed one of our tweets. Another conclusion we found was that the campaigns which had restrictions (San Antonio, Houston, Dallas, Austin) had 13 more followers than the Texas Campaign which had no restrictions. This proved that having a narrow advertisement works better than a broad advertisement on Twitter. This information can be essential for future advertising on Twitter for other small campaigns like ours, and for other businesses. With more engagement on Twitter or other social media platforms, we could be helping these generations overcome substance abuse and addiction.

Development of evidence-based alcohol intervention for adults with driving while intoxicated offenses.

Maria Silvaz and Charles W. Mathias, Ph.D.

Background: Among the U.S., San Antonio, Texas is one of the highest ranking metropolitan areas with driving while intoxicated (DWI) arrests. After a DWI arrest, DWI offenders are mandated to abstinence from alcohol while awaiting trial. However, there continues to be high rates of continued alcohol use, DWI recidivism and few resources available for those having difficulty reducing their alcohol use. This study examined the feasibility and efficacy of implementing alcohol screening, brief intervention, and referral to treatment a program for adults awaiting trial for DWI.

Methods: DWI offenders attended a pretrial orientation typically within 2 weeks of being arrested. At this orientation they were approached for participation and those enrolled completed a computer-assisted screening, brief intervention and referral to treatment (SBIRT) program about alcohol. An outcome of the screening assessment was to quantify their level of alcohol risk and those who were greater than low risk were referred for treatment. One week later, their plans for enrolling in treatment were assessed and then their compliance with pretrial conditions was assessed prospectively. Finally, some referrals were accepted into contingency management and cognitive behavioral therapies delivered by our program. Tests of outcomes were compared.

Results: A significant proportion of those referred for treatment either had engaged or had plans to engage in treatment one week after the initial SBIRT session. Those who had engaged or planned to engage had fewer pretrial violations than those who were not pursuing recommended follow-up care. With the start of contingency management and cognitive behavior therapy, it is too early to test outcomes, although feasibility tests can be conducted.

Discussions: SBIRT appears to improve pretrial compliance for those who pursue treatment recommendations. This ongoing study continues to follow evidence of long-term gains and added benefit of contingency management and cognitive behavior therapy treatment modalities for DWI offenders.

Host Genetic Determinants in Autoimmunity

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Background: Infection with HIV leads to CD4+ T-cell destruction and eventually disease progression towards AIDS. However, certain variations in specific autoimmune base pairings have been associated with different rates of disease progression and quantitative variables such as viral load, CD4 count, etc. Aside from knowing which SNPs (Single Nucleotide Polymorphisms) are associated with disease acceleration, the phenotypic effect that these SNPs have on autoimmunity is unknown.

Methods: For our study, we found the genotypes for SNPs that have shown to have an association with autoimmunity and immune traits in general. By taking various DNA samples from over 4,000 people, we ran real-time PCR to find the specific genotype for each desired SNP. We classified the data as homozygous wildtype, heterozygous, or homozygous mutant based on whether the SNP had a fluorescent marker illuminate.

Results: The dataset for this project is pending since the genotyping is still ongoing, but results from a previous study have demonstrated that the Tri-Service AIDS Cohort (TACC) can be useful in a genotyping study due to its diverse nature. We plan to evaluate the new genotype dataset in a way similar to the previous study, in which a genetic score was assigned based on the genotypes found in 5 SNPs different from the current 10. The scores of <0 , $=0$ and >0 represent association with detrimental, neutral and beneficial HIV outcomes, respectively. The scores from each individual SNP are summed and that overall score will describe the beneficial or detrimental nature of their unique genetics. Once genotyping of the current 10 SNPs is complete, we will utilize this strategy to analyze our results.

Conclusion: With this data, we plan to quantify the genotype of each person into two qualitative categories representing detrimental or beneficial HIV outcomes based on the genetic score. Once completed, further associations will be analyzed in order to assess the magnitude each SNP possesses with respect to HIV outcomes.

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Genotyping Chromosome 18 Copy Number Changes

Isabella Torres, Patricia Heard, Jannine Cody Ph.D

Introduction: Chromosome 18 spans about 78 million DNA base pairs and represents approximately 2.5 – 2.7 percent of the total human DNA. Chromosome 18 contains approximately 260 genes that provide instructions for making proteins. About 1 in 40,000 babies are born with an abnormality of chromosome 18 that involves a deletion or duplication of one or more genes. Each of the conditions of chromosome 18 can result in medical or developmental issues in an individual which can vary in severity. How an individual is affected depends on where the deletion or duplication of the chromosome is located and what genes are involved. In 18p-, there is a missing piece of the p arm (short arm) of the chromosome. In 18q-, there is a missing piece from the q arm (long arm) of the chromosome. If the missing piece is close to the centromere, then it is known as proximal. If the missing piece is close to the end of the chromosome, then it is known as distal. In addition, the chromosome can form a ring when the end of one of the chromosome arms fuses with the end of the other arm, called Ring 18. In most cases of Ring 18, material from both ends of the p arm and the q arm is lost. In Trisomy 18, there are three copies of the chromosome instead of the usual two copies in every cell. Tetrasomy 18p, an extra chromosome is present. This extra chromosome is made up of two copies of the p arm. There are a total of four copies of the short arm of chromosome 18.

Methods: Custom oligonucleotide arrays were produced by Agilent Technologies for comparative genomic hybridization (CGH) analysis of chromosome 18 copy number. These chromosome 18 zoom arrays were designed using the Agilent array software. This allowed us to generate very high-resolution chromosome 18 copy number data for the individuals in our study. CGH uses a two-sample comparative method in which the test (or patient) sample is assessed in comparison to a reference sample. The two DNA samples are labeled with different fluorophores, then mixed together and allowed to competitively hybridize to the oligonucleotides on the array slide. The reference sample is from the opposite sex as the test sample thus providing an internal copy number control. Each array was scanned using the Agilent laser scanner. Those data were then analyzed using the CGH Analytics 3.4.27 software. Data points were analyzed in continuous groups of eight probes and log₂ ratios of sample DNA were compared to control DNA. Arrays were normalized to a median log₂ ratio of zero, except for the X and Y chromosomes. Breakpoints were determined to be between the ends of the array features on either side of the deletion breakpoint.

Results: The DNA from 8 individuals was assessed; 2 children with 18p-, 1 Ring18, and 5 with 18q-. Two of the people with 18q deletions were determined to have an interstitial deletion; a type of deletion that occurs from the interior of a chromosome arm. The other 3 with an 18q deletion showed a terminal deletion; a type of deletion that involves the end of the chromosome arm.

Conclusion: Genotyping using DNA microarray allowed us to determine the genetic make-up; pinpointing the exact genes that are involved in these gene copy number changes deletions. This is necessary step in order to provide gene specific prognosis and treatment information to the affected families.

The Use of Analytical Ultracentrifugation to Determine Partial Specific Volume and Frictional Ratio of Protein

Jessica Tuholsky, Christopher Nash, Ryan Cao, Virgil Schirf, Borries Demeler, Ph.D.

Understanding the structure of specific proteins is a necessary aspect of performing experiments with the said proteins. One technique for determining the structure of a protein is with bead modeling software, which estimates the hydrodynamic properties of a macromolecule in solution based on the crystal structure of the molecule. The goal of this study was to compare the performance of several bead modeling algorithms implemented in the UltraScan SOMO software by providing reference data for the software from analytical ultracentrifugation experiments. A secondary goal was to validate a new analytical ultracentrifugation analysis method for fitting partial specific volumes based on known protein molecular weights and to check if the effect of sodium chloride on the partial specific volume and frictional ratio can be determined. Five different proteins were prepared in a 20 millimolar TRIS 2 millimolar EDTA 8 pH buffer. The proteins chosen were those with well-known protein structures and easily accessible crystal structures. The proteins used were Sigma-Aldrich cytochrome-C from horse heart, carbonic anhydrase from bovine erythrocytes, lysozyme chloride from chicken egg whites, chymotrypsinogen-A from bovine pancreas, and ribonuclease-A from bovine pancreas. Before beginning analytical ultracentrifugation experiments, the proteins were dialyzed against the TRIS buffer using dialysis membrane tubing. The buffer was changed once to ensure that the proteins were as pure as possible. In order to measure the effect of salt on partial specific volume and frictional ratios, the proteins were prepared with seven different sodium chloride concentrations from 0 millimolar to 150 millimolar. Spectrophometric analysis was then performed to determine optical density of each sample. A thermo Scientific Genesys 10S UV-Vis Spectrophotometer was used to measure the ultraviolet-visible absorbance profiles of a standard 1 milligram/milliliter stock of each protein. Once the optical density of the stock had been determined, UltraScan2's Global Extinction Fit program was employed to find the optical densities of the substance at each wavelength at 1 molar by producing a molar extinction curve. These data were applied to dilute the proteins to concentrations where the optical density would be 0.3 and 0.9. The 0.3 and 0.9 optical density concentrations were necessary to run in the analytical ultracentrifuge using the optical detection capabilities; any absorbance above 1.2 optical density was usually too high for accurate detection in the analytical ultracentrifuge. The proteins were run in the analytical ultracentrifuge set to measure at wavelength 280 nanometers with the 0.3 optical density concentrations in the reference channels and the 0.9 optical density concentrations in the sample channels. After all of the proteins had been run at each sodium chloride concentration, the results were analyzed using the UltraScan3 program with Lims3 queuing system to submit highly computationally demanding jobs to supercomputers around the country. The goal of the analysis was to determine the partial specific volume and frictional ratio of each protein at each salt concentration. The first analysis was a series of 2DSA analyses for fitting time and radially invariant noise and fitting meniscus. Then, 2DSA iterative refinement and Monte Carlo analyses were evaluated. UltraScan's van Holde-Weischet program used the results of the iterative refinement to determine sedimentation coefficient ranges for the Monte Carlo analysis. The final analysis was a PCSA with Monte Carlo iterations; in which, the molecular weight known from peptide sequence is used as a constraint to fit the data to obtain a frictional ratio and partial specific volume. The studies are ongoing, but the data seems to suggest that as the concentration of sodium chloride in a protein solution increases, the partial specific volume of the protein decreases and the frictional ratio of the protein are expected to increase.

New Curative Strategy for Chronic Granulomatous Disease

Thomas Vargas, Cang Chen, Xiaoling Zhao, and Senlin Li

Chronic granulomatous disease (CGD) is a type of genetic disease in which those who are afflicted develop a form of immunodeficiency. CGD is caused by genetic mutations in one of the five subunits gp91phox, p67phox, p47phox, p40phox and p22phox of the NADPH oxidase (nicotinamide adenine dinucleotide phosphate-oxidase). NADPH oxidase is a membrane-bound enzyme complex that catalyzes superoxide production. CGD prohibits the ability of neutrophils and other phagocytes to produce superoxide and other oxidants that kill bacteria and fungi. Therefore, victims of CGD are generally susceptible to these infections. Frequent and potentially fatal infections will often reoccur in patients every three or four years. Patients afflicted with this disease may develop infections of the skin, liver, lymph nodes, and especially the lungs and intestinal tract. This disease typically occurs in 1 out of 200,000-250,000 people.

Current methods of patient care involve an aggressive administration of antibiotics and prednisone, targeting symptoms of the disease, but not the disease itself. Antibiotics will suppress bacterial infections while prednisone will suppress some inflammation. As neutrophils and other phagocytes are derived from hematopoietic stem cells (HSCs) and HSC transplantation (HSCT, also called bone marrow transplantation) is a proven clinical practice for decades, HSCT combined with gene therapy has been considered to be a curative strategy for CGD. However, two problems will need to be resolved to make this strategy successful. Firstly, conventional pre-transplant conditioning regimens are done using irradiation and/or chemotherapeutic agents and cause severe adverse effects. Secondly, retroviral random integrations into the genome can be tumorigenesis. Thanks to many recent advances in biomedical science and our vigorous efforts, we have found potential solutions for both problems.

For the first problem, we have been developing a novel HSCT method that is irradiation and chemotherapy-independent and essentially free of side effects. For problem two, it has now become possible to specifically target a genetic sequence using the latest CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/Cas9 (CRISPR-associated protein-9 nuclease) technology. Using CRISPR/Cas9 gene editing technology, we will correct the mutated genes by specific targeting and editing of the genome of HSCs. Since about two-thirds of all CGD patients have developed the disease as a result of a mutation in the gene gp91phox, we will focus on this gene mutation first. We will either target the DNA sequence on which gp91phox lies to correct the mutated gene or target the AAVS1 safe site to add the normal gp91phox gene there for expression. The corrected HSCs will be transplanted back to the same patients in order to repopulate their blood cells including neutrophils and other phagocytes that have been corrected for the mutations and express functional gp91phox. These cells will produce superoxide and can kill invading microorganisms, providing normal immune defenses. CGD has been replicated in murine subjects genetically modified not to express functional gp91phox protein, thereby exhibiting similar symptoms that are visible in human subjects. The study will be performed in this gp91phox knockout model.

If we are able to successfully transfer a functional gp91phox gene into the hematopoietic stem cells of a patient, gp91phox mutation-caused CGD cases could be cured. Similarly, we can also pioneer methods to cure all other cases of chronic granulomatous disease that constitute the remaining third of patients, those including mutations on the p67phox, p47phox, p40phox or p22phox genes. Additionally, I have been involved in a parallel project conducted in the lab to develop hematopoietic stem cell (HSC) transplantation-based macrophage/microglia-mediated delivery therapy for Parkinson's disease. I participated in scoring the video records of mouse behavioral tests such as the stepping test, tail suspension test, and novel object recognition test. Furthermore, I learned basic lab skills and practiced lab routines such as autoclaving and aliquoting.

Maternal Obesity Dependent Alterations to 11 β HSD Obesity in the Human Placenta

Iman Wallace, Calais Prince, Alina Maloyan, Leslie Myatt

Background: The human placenta stores a supply of nutrients to the fetus that is essential to its growth, including water, electrolytes, glucose, proteins, amino acids, lipids and triglycerides, and other vitamins. Cortisol (hydrocortisol), a steroid hormone released by the adrenal cortex acts as a stress hormone in response to stressful conditions. Cortisone is the inactive version of the cortisol steroid hormone. 11 β Hydroxysteroid Dehydrogenase Type 1 (11 β HSD1) converts cortisol into its inactive version cortisone. 11 β Hydroxysteroid Dehydrogenase Type 2 (11 β HSD2) activates cortisone into cortisol.

Methods: Following delivery by C-section at term with no labor, placental tissues were immediately collected from female (n= LN-F, OB-F), and male (n=LN-M, OB-M) fetuses. Random sample collection from the placenta was used to quantify protein abundance using Western Blotting. To test the hypothesis, 11 β HSD1 and 11 β HSD2 were quantified by and Western Blotting. 3 of each LN-F, OB-F, LN-M, and OB-M were tested in each Western Blot. Western Blots were performed over a period of three weeks: (1) 11 β HSD1, (2) improved 11 β HSD1, (3) 11 β HSD2. Samples were run on SDS/PAGE, transferred, and blocked with appropriate antibodies. An additional Actin (multi-functional protein found consistent throughout most tissue) Western Blot was performed as a control after each Western Blot.

Results: 11 β HSD1 levels were too low to analyze and could not be detected in our samples. 11 β HSD2 was decreased in placentas from OB women when compared to LN women (p=0.0002). When comparing male fetuses and female fetuses, the gender did not show to affect the 11 β HSD levels. ProBDNF expression was decreased in OB-M when compared to LN-M and OB-F (p=0.04, 40% of LN-M; p=0.03, 35% of OB-F). Expression of the inactive, mature BDNF monomer was greater in OB-M when compared to OB-F (p=0.005, 180% of OB-F). Conversely, expression of the active, mature homodimer to total mature BDNF was lower in OB-M when compared to OB-F (p=0.01, 31% of OB-F). Total TRKB expression was decreased in OB-F when compared to LN-F (p=0.003, 47% of LN-F). TRKB autophosphorylation at tyrosine 515 was decreased in OB-F when compared to LN-F (p=0.01, 50% of LN-F) and increased in OB-M when compared to OB-F (p=0.02, 100% of OB-F).

Conclusion: We were unable to detect 11 β HSD1 in the placenta homogenates. This corresponds with previous data that shows 11 β HSD1 localized in intermediate trophoblasts and vascular endothelium. The data also suggests that maternal obesity decreases placental 11 β HSD2. As the protective barrier from maternal cortisol, decreased placental 11 β HSD2 with maternal obesity suggests that the barrier is weakened and therefore increased cortisol levels.

A Novel Neuroprotective Therapy for Parkinson's Disease

Christina Zhu, Cang Chen, and Senlin Li, M.D.

Parkinson's disease (PD) is a chronic and progressive neurodegenerative disorder that affects thousands of patients each year. A patient diagnosed with PD is characterized by the deterioration of his/her motor skill, leading to tremors and problems with coordination and movement. PD is caused by neurodegeneration and eventual death of dopaminergic neurons located in the substantia nigra. Neuroprotective therapy to block the disease process is desired but unavailable. Current treatments, only managing symptoms, are effective initially, but will lose effectiveness with the disease progression. We have been working to develop a neuroprotective therapy for PD in the last few years. Glial cell line-derived neurotrophic factor (GDNF) is the most potent neurotrophic molecule for protection of the dopamine (DA) neurons affected in PD. However, GDNF cannot properly cross the blood-brain barrier (BBB), so systemic delivery is ineffective. Focal injection of the GDNF protein is not so effective either due to its limited diffusion in brain tissue. We have developed a novel technology with the potential to resolve the limitations of direct brain delivery approaches, namely hematopoietic stem cell (HSC) transplantation-based macrophage/microglia-mediated GDNF delivery. This unique approach takes advantage of the well-known macrophage property of homing to degenerating central nervous system sites in proximity to damaged neurons, incorporates our powerful macrophage-specific synthetic promoters (MSPs), and capitalizes on the long-standing clinical experience with HSC transplantation (HSCT), as well as recent advances in HSC gene therapy. The clinical scenario of this novel therapy is that autologous HSCs are mobilized from bone marrow, isolated from peripheral blood by apheresis, and then transduced *ex vivo* with a lentiviral vector carrying the *GDNF* gene. The transduced HSCs are infused into the patient after pre-conditioning, resulting in engraftment of the transplanted HSCs that will form various blood cell lineages. The therapeutic gene will be expressed at high levels only in cells of the monocyte/macrophage lineage because it is under MSP control. The macrophages will infiltrate the brain and become microglial cells, which accumulate in the nigrostriatal system where neurodegeneration is focused in PD patients. These microglial cells will secrete GDNF protein and make the trophic factor accessible to surrounding neurons that are affected in the patients. Using our approach in various mouse models of PD, we demonstrated that genetically engineered HSC-derived macrophages accumulated selectively in diseased sites and macrophage-mediated neurotrophic factor delivery dramatically ameliorated degeneration of tyrosine hydroxylase (TH)-positive dopaminergic neurons of the substantia nigra and TH+ terminals in the striatum, stimulated axon regeneration, and markedly improved their motor and non-motor dysfunctions, without apparent adverse effects. I have been involved in this study by scoring the video records of mice behavioral tests such as the stepping test, tail suspension test, and novel object recognition test. However, the random integrations of lentiviral vectors might lead to unwanted side effects. Gene therapy through genomic editing would provide a high possibility to put the therapeutic gene into a safe site of the genome. We will utilize CRISPR/Cas9 gene editing technology to incorporate *GDNF* gene into the safe harbor ROSA26 of the mouse genome and then test the therapeutic effect in mouse models for PD as described above. Our experiment will likely hinder the advancement of Parkinson's by offering a novel way to protect the neurons, targeting the cause of Parkinson's disease rather than just the symptoms.

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