postulate that mimicking LH GABAergic activity will produce its previously demonstrated anxiolytic effects. DISCUSSION/SIGNIFICANCE OF IMPACT: Identifying the important role for a reward-oriented feeding center in the brain in producing antipsychotic weight gain will allow a more comprehensive, ethologically sound approach to behavioral modification therapy in these patients. It will lend mechanistic credence to weight control therapies which have used token economy, opioid antagonism, and other inhibition-promoting therapies. This study will also increase the validity for testing further the use of selective serotonin agonists which prevent weight gain such as lorcaserin.

2153

Innovative 3D printed intravaginal rings for contraception and HIV prevention

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OBJECTIVES/SPECIFIC AIMS: The long-term goal of this project is to develop a cost effective 3D printed multipurpose intravaginal ring (IVR) to prevent against unintended pregnancies and infectious diseases.Our goal is to develop a female-controlled method for prevention using innovative IVRs. METHODS/STUDY POPULATION: In vitro and in vivo characterization. RESULTS/ANTICIPATED RESULTS: Controlled and fine-tuned release kinetics 100% drug release from 3D printed IVRs compared with 10%–15% with traditional injection molded IVRs cost-effective engineering of multipurpose IVR with CLIP 3D printing technology. DISCUSSION/SIGNIFICANCE OF IMPACT: If successful, this project will revolutionize the engineering of IVRs and will have a global impact on human health. Not only we will help save millions of women around the world but also millions of children that are infected by their HIV-positive mothers through gestation or breast feeding.

2169

Hydrogen bonding and water accessibility changes upon expansion of PolyQ tracts in ataxin-2 and ataxin-3

Jingran Wen, Daniel Scoles and Julio C. Facelli

OBJECTIVES/SPECIFIC AIMS: Polyglutamine (polyQ) neurodegenerative diseases, associated with the unstable expansion of polyQ tracts, are devastating diseases for which no treatments exist. Moreover, most drug discovery attempts have been hindered by the lack of understanding on the relevant pathogenic mechanisms. Here, using previously reported 3D protein predicted structures of ataxin-2 and ataxin-3, we analyze the effect of polyQ enlargement on hydrogen bonding and water accessibility patterns as a possible mechanism for pathogenesis thought enhanced protein aggregation. METHODS/STUDY POPULATION: Using the I-TASSER predicted structures of ataxin-2 and ataxin-3 with different numbers of glutamine repeats representing polyQ lengths characteristic of both normal and pathological tracts (*Journal of Biomolecular Structure and Dynamics*, 2016: 1–16), we identified hydrogen bonds (HBs, UCSF Chimera FindHBond module) and calculated solvent-accessible surface areas (SASA, DSSP program) for the polyQ tracts available in the 3D structures. RESULTS/ANTICIPATED RESULTS: The identified HBs were analyzed as the function of the number of glutamines in the polyQ tracts and characterized as those intra-polyQ and exter-polyQ, respectively. The SASA of the polyQ region was also studied as the function of the polyQ tract length. DISCUSSION/SIGNIFICANCE OF IMPACT: The results obtained here indicate that polyQ regions increasingly prefer self-interactions, which consistently can lead to more compact polyQ structures. The results strongly support the notion that the expansion of the polyQ region can be an intrinsic force leading to self-aggregation of polyglutamine proteins and suggest that the modulation of solvent-polyQ interactions could be a possible therapeutic strategy for polyQ diseases.

2173

Investigation of sAC signaling reveals new therapeutic targets for cancer cell metabolism

Jenny Wang and Jonathan Zippin

OBJECTIVES/SPECIFIC AIMS: The soluble adenylyl cyclase (sAC) is a noncanonical source of cAMP in mammalian cells. sAC is an ATP/bicarbonate ion sensor, whose activity responds to intracellular signals such as pH changes and metabolism. Unlike the more traditionally studied transmembrane adenylyl cyclase, sAC is not tethered to the cell membrane and instead is found in subcellular microdomains like the mitochondria and nucleus. In particular, sAC localization in the mitochondria has been implicated in oxidative phosphorylation and mitochondrial metabolism. Specific changes in sAC microdomain localization have diagnostic utility in a wide variety of cancers, namely melanoma. We have recently found that loss of sAC leads to tumorigenesis and a Warburg/cancer-like metabolic phenotype, consisting of an activated flux through glycolysis, increased lactate production, and dependence on glucose metabolism. In addition, computational analysis of the metabolomics profile of sAC null cells suggests an increased flux through serine synthetic pathways. We hypothesized that specific sAC microdomains are responsible for this cancer-like metabolic state. METHODS/STUDY POPULATION: We have established oncogenic SV40 large T antigen and HPV16-E6 expressing mouse embryonic fibroblasts lacking sAC expression (SV40 KO and E6 KO, respectively). Using these parental lines, we reintroduced sAC by targeting the protein to specific microdomains. sAC was either driven into the mitochondria (mito-sAC) or was driven into all possible microdomains (WT sAC). Single clones were generated and sAC expression was confirmed by Western analysis. Stable cell lines were evaluated for mitochondrial metabolism, glucose sensitivity, and serine sensitivity. RESULTS/ANTICIPATED RESULTS: We found that reintroduction of WT sAC into sAC null cells rescued sensitivity to glycolytic inhibition compared with control cells ($p < 0.01$). The effect was not dependent on the method of immortalization as it was seen in both SV40 and E6 KO cell lines. sAC activity was not directly proportional to expression suggesting that additional regulatory pathways exist. Interestingly, targeted delivery of sAC to the mitochondria was not as effective in rescuing glucose sensitivity as untargeted delivery of sAC into all possible microdomains. Therefore, even though mitochondrial sAC is known to influence metabolism, our data suggests that the nonmitochondrial isoform is most important for cancer cell metabolism. Although metabolomics analysis suggested that serine synthetic pathways are activated in sAC null cells, there is no evidence to suggest that serine is required for sAC null cell growth. Neither inhibition of serine synthesis nor serine starvation differentially affected the growth of sAC null cells compared with WT sAC. DISCUSSION/SIGNIFICANCE OF IMPACT: These data suggest that the Warburg metabolic phenotype in sAC null cells is regulated by specific sAC microdomains. By targeting sAC to specific microdomains, we can further distinguish the role of sAC localization in cellular metabolism. Cancer cells have been shown to exhibit altered metabolic circuitry of pathways like glycolysis, which allow them to adapt to increased metabolic demands of cellular proliferation and waning environmental resources. Beyond helping us improve the use of sAC immunolocalization as a cancer diagnostic, a better understanding of sAC microdomains in transformed cells will help us understand how this signaling pathway is important in cancer. Pharmacologic manipulation of sAC signaling may represent a new cancer therapeutic strategy.

2174

In silico prediction of NS1 structure and influenza A virus pathogenesis

Joshua Klonoski and Julio C. Facelli

OBJECTIVES/SPECIFIC AIMS: This poster presents preliminary results of using in silico approaches to predict a priori, based on sequence alone, the pathogenesis of novel influenza A virus. METHODS/STUDY POPULATION: Here we analyzed the structure of the NS1 protein of 11 strains of well characterized influenza A virus with known pathogenesis, reported in the literature as LD50 values, and published sequences. We performed homology comparison of these sequences using the ExPASy SIM alignment tool for protein sequences and then predicted their 3D structures using the I-TASSER method for protein structure prediction. We retained the best 20 I-TASSER models for the NS1 sequences considered here and compared their structures with that of the X-ray crystallographic structure of the NS1 protein in the A/blue-winged teal/MN/993/1980 (H6N6). The average RMS between this experimental structure and the best 20 I-TASSER models was used as a measure of structural similarity between the 3D structures among the proteins. RESULTS/ANTICIPATED RESULTS: The sequence homology shows modest correlation between sequence and pathogenicity. Linear correlations with R values as large as 0.6 where observed for the full sequence homology and the homology of the RBD domains of the proteins. The correlations with the other protein domains were significant lower. We did not found overall correlation between the 3D structures and pathogenesis of all the variants considered here, but the initial results suggest that correlations do exists for different subgroups of viruses. In future work we will use advanced data mining methods to better understand clustering and correlation between structure and pathogenesis. DISCUSSION/SIGNIFICANCE OF IMPACT: The results presented in this poster demonstrate, as proof of concept, the use of in silico approaches to determine pathogenesis of viruses with substantial impact on human health. The ability of computationally predicting pathogenesis of rapidly mutating viruses

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can be an effective way to accelerate the development prevention strategies because computational methods are relatively inexpensive and much more scalable than in vivo approaches.

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Comparison of liquid Versus dry aerosol drug delivery in a 3D printed avian trachea and mainstream bronchi model

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OBJECTIVES/SPECIFIC AIMS: This study investigates the process configuration parameters involved in targeted drug delivery to the avian respiratory system. Previously, direct intratracheal aerosol delivery in an avian model using a commercial atomizer was found to result in delivery of a high portion of the total dose into one lung lobe. We hypothesize that controlling process configuration will decrease the asymmetric distribution. METHODS/STUDY POPULATION: A 3D printed model of an avian trachea and mainstream bronchi was constructed to create a representative model for direct instillation of aerosols. Construction of the model respiratory tract included the trachea and the first mainstream bronchi bifurcation to measure left/right (L/R) distribution of aerosol delivered. Both liquid aerosol delivery (LAD) using a commercial atomizer and dry aerosol delivery (DAD) using a custom-built dry powder insufflator device were tested. Two experimental variables were controlled: (1) retraction distance from the carina and (2) centering of device shaft in the lumen of the trachea. Measurement of device efficiency (dose delivered to the 3D model at as fraction of total dose), aerosol delivery efficiency (dose captured at L/R bifurcations as a fraction of total dose), and aerosol lateralization (L/R) was conducted. RESULTS/ANTICIPATED RESULTS: The aerosol delivery efficiency for both LAD and DAD devices [73.9% (95% CI: 68.2–79.2) and 73.4% (95% CI: 55.5–91.3), respectively] did not have an appreciable difference. However, the LAD device had a higher efficiency as compared with the DAD device. The L/R distribution for the DAD device was found to be highly dependent on both retraction distance and shaft centering. Appreciable improvement in the L/R distribution was seen using the DAD device by increasing the retraction distance distal to the carina. DISCUSSION/SIGNIFICANCE OF IMPACT: The use of targeted drug delivery to treat pulmonary pathogens requires a careful design, manufacture, and therapeutic positioning of devices. In particular, clinically relevant animal models and treatment regimes requires a sound understanding of the physical processes controlling aerosol distribution in the respiratory system. By using a simulated respiratory model, many of the physical parameters of drug delivery can be tested before using a live animal model. This is especially important from an animal welfare perspective as well as an animal subject availability aspect.

2194

Effects of anoxia on viability and differentiation of human cardiosphere-derived cells

Michael Khanjyan, Vien Nguyen, Eric Kazangian, Shane Browne, Kevin Healy, Kurosh Ameri and Yerem Yeghiazarians

OBJECTIVES/SPECIFIC AIMS: A major limitation of cardiac stem cell transplantation following myocardial infarction (MI) is poor retention of cells in the ischemic microenvironment. Our study aims to better understand and promote the survival and differentiation of human cardiosphere-derived cells (hCDCs) in anoxia, a feature of infarcted myocardium. METHODS/STUDY POPULATION: We previously demonstrated that TGFβ1 and heparin-containing hydrogels (TH-hydrogel) can promote murine CDC survival. In this study, hCDCs were incubated in either normoxia or anoxia for 8 hours with and without TH-hydrogel. In addition, hCDCs without TH-hydrogel were assessed in 16 hours of anoxia. Following incubation, hCDCs were assayed for viability using calcein dye and immunostained for CD31, a marker of endothelial differentiation. RESULTS/ANTICIPATED RESULTS: hCDCs incubated for 8 hours in anoxia in both models equally demonstrated increased survival up to 30% when compared with cells incubated in normoxia. However, in contrast to hCDCs alone, hCDCs with TH-hydrogel additionally demonstrated increased differentiation into endothelial cells in both anoxia and normoxia. We found that hCDCs alone were able to upregulate CD31 only when subjected to 16 hours of anoxia. DISCUSSION/SIGNIFICANCE OF IMPACT: We demonstrate a new, previously unknown response of hCDCs to anoxia. This induces increased viability and differentiation of hCDCs into endothelial cells. The differentiation in anoxia was time dependent and could be expedited with use of TH-hydrogel. Anoxic preconditioning of hCDCs together with the TH-hydrogel system may improve the therapeutic potential of stem cell transplantation following MI.

2215

Neuropilin-2 is expressed by activated alveolar macrophages and negatively regulates allergic airway inflammation

Timothy P. Moran, Robert M. Immormino, Hideki Nakano, David Peden and Donald N. Cook

OBJECTIVES/SPECIFIC AIMS: Allergic asthma is a chronic lung disease driven by inappropriate inflammatory responses against inhaled allergens. Neuropilin-2 (NRP2) is a pleiotropic transmembrane receptor expressed in the lung, but its role in allergic airway inflammation is unknown. Here, we characterized NRP2 expression in lung immune cells and investigated the effects of NRP2 deficiency on airway inflammation. METHODS/STUDY POPULATION: NRP2 expression by lung immune cells from NRP2 reporter mice was determined by flow cytometry. NRP2 expression by human alveolar macrophages (AM) from healthy individuals was determined by mRNA analysis and flow cytometry. Airway inflammation in NRP2-deficient mice was assessed by bronchoalveolar lavage (BAL) cytology and inflammatory gene expression in lung tissue. RESULTS/ANTICIPATED RESULTS: NRP2 expression in lung immune cells was negligible under steady-state conditions. In contrast, inhalational exposure to lipopolysaccharide (LPS) adjuvant dramatically induced NRP2 expression in AM, as 63.3% of AM from LPS-treated mice were NRP2+ compared with 1.5% of AM from control mice. Ex vivo treatment of human AM with LPS resulted in a 1.5-fold and 2.6-fold increase in NRP2 mRNA and surface protein expression, respectively. Compared to littermate controls, NRP2-deficient mice had greater numbers of BAL leukocytes and increased lung expression of the T helper type 2 cytokines IL-4 and IL-5. Furthermore, NRP2 deficiency resulted in stochastic development of allergic airway inflammation, as spontaneous airway eosinophilia was detected in 25% (2/8) of NRP2-deficient mice compared with 0% (0/8) of littermate controls. DISCUSSION/SIGNIFICANCE OF IMPACT: NRP2 is expressed by activated human and murine AM and suppresses the spontaneous development of allergic airway inflammation. These findings suggest that NRP2 may play a key role in allergic asthma pathogenesis, and could prove to be an important therapeutic target in patients with asthma and other allergic diseases.

2217

A transgenic retinitis pigmentosa zebrafish model for drug discovery

Logan Ganzen, Chi Pui Pang, Mingzhi Zhang, Motokazu Tsujikawa and Yuk Fai Leung

OBJECTIVES/SPECIFIC AIMS: Retinitis pigmentosa (RP) is a hereditary retinal degeneration disease that affects ~1 in 4000 individuals globally, and there are currently no effective treatment options available. In order to identify potential drug treatments, we optimized our existing a behavioral assay around a transgenic zebrafish carrying a truncated human rhodopsin transgene [Tg(rho:Hsa.RH1_Q344X)]. This line was also crossed with the Tg(-3.7rho:EGFP) reporter for rod visualization. The Q344X larvae experiences significant rod photoreceptor death by 7 days postfertilization (dpf) (Nakao *et al.*, 2012). METHODS/STUDY POPULATION: To assess the vision of the Q344X zebrafish, the VMR assay was run under a dim-light condition based on recorded rod b-waves in larval fish (Moyano *et al.*, 2013) and the minimum cone activation threshold in mice (Cachafeiro *et al.*, 2010). Specifically, Q344X and control larvae at 7 dpf were placed into a 96-well plate and acclimated to a dim-light source (1.802e-05 μ W/cm^2 at 500 nm) for 1 hour. The VMR was tracked and quantified during light offset. The total distance traveled was averaged and analyzed at 1 second poststimulus. Retinas were dissected from Q344X and control larvae and whole-mounted to validate the rod degeneration in the Q344X model. RESULTS/ANTICIPATED RESULTS: We found that the Q344X larvae displayed an attenuated VMR (0.121 ± 0.041 cm) to the dim-light offset as compared with the control larvae (0.2751 ± 0.038 cm) (two-sample *t*-test; *p*-value = 4.619e-14, n = 19). Analysis of whole-mounted retinae indicated significant rod degeneration at 7 dpf compared with controls (control: 87 rods/retina, Q344X: 9.3 rods/retina, Welch two-sample *t*-test, *p*-value = 1.4e4). It is unlikely that the cones of the zebrafish contributed to this VMR since the light intensity of the assay was below the cone detection threshold of mice. As the only apparent difference between the 2 groups of larvae is significant rod degeneration, it can be concluded that the behavioral phenotype was a result of

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