BASIC SCIENCE/METHODOLOGY

2079

Updates to the documentation system for R
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OBJECTIVES/SPECIFIC AIMS: This research seeks to create a next generation documentation system that exists independent of but is complimentary to the packaging system in R. The new documentation can be manipulated programmatically as well as with all R objects. It also implements multiple translators for creating documentation from different sources, including documentation pages written in latex and code comments.

METHODS/STUDY POPULATION: This work is based on input from the R Documentation Task Force, which is a working group, supported by the R Consortium and the University of Utah Center for Clinical and Translational Science, consisting of R Core developers, representatives from the R Consortium member companies and community developers with relevant interest in documentation. An abstraction of the documentation currently in use was created and extended. This abstraction was translated to a class system in R so that documentation can be stored and manipulated in R.

RESULTS/ANTICIPATED RESULTS: The class system representing the documentation and the tools for creating the translators are currently being implemented in R. A preview of the system is scheduled to be available at the time of the conference.

DISCUSSION/SIGNIFICANCE OF IMPACT: Good documentation is critical for researchers to disseminate computational research methods, either internally or externally to their organization. This work will facilitate the creation of documentation by making documentation immediately accessible and promote documentation consumption through multiple outputs which can be implemented by developers.

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Interleukin 4-induced protein 1 as a biomarker and treatment option in multiple sclerosis
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OBJECTIVES/SPECIFIC AIMS: The overall objective of this proposal is to establish and modulate the inflammatory profile of individuals across the spectrum of multiple sclerosis (MS), with a focus on determining the potential of interleukin 4-induced protein 1 (IL4I1) as a possible marker of progression and modulator of inflammation in human blood samples.

METHODS/STUDY POPULATION: The proposed experimental approach involves isolating plasma and peripheral blood mononuclear cells (PBMCs) from individuals across the spectrum of MS phenotypes, and analyzing these samples primarily by quantitative polymerase chain reaction (qPCR) and enzyme-linked immunosorbent assay (ELISA) methods. Specifically, study groups include: (1) actively relapsing-remitting MS (a-RRMS), (2) non-actively relapsing-remitting MS (n-RRMS), and (3) active secondary-progressive MS (SPMS), (4) other autoimmune diseases (OAD), (5) healthy controls (HC).

RESULTS/ANTICIPATED RESULTS: We expect that IL4I1 treatment increases regulatory cytokine (e.g., IL10, TGFβ) expression while decreasing Th1 and Th17-derived cytokines (IFNγ, IL17), as well as increasing relative composition of regulatory cells (Th2, Treg, M2) as compared with Th1 and TH17-derived cytokines (IFNγ, IL17), as well as increasing relative composition of regulatory cells (Th2, Treg, M2) as compared with Th1 and TH17-derived cytokines (IFNγ, IL17), as well as increasing relative composition of regulatory cells (Th2, Treg, M2).

PATED RESULTS: The class system representing the documentation and the tools for creating the translators are currently being implemented in R. A preview of the system is scheduled to be available at the time of the conference.

Significance/Impact: Good documentation is critical for researchers to disseminate computational research methods, either internally or externally to their organization. This work will facilitate the creation of documentation by making documentation immediately accessible and promote documentation consumption through multiple outputs which can be implemented by developers.

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Antipsychotic-induced weight gain arises, in part, from alteration of feeding circuitry in the lateral hypothalamic area
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OBJECTIVES/SPECIFIC AIMS: To demonstrate that olanzapine recapitulates the effect of increased lateral hypothalamic (LH) GABAergic activity in the DRN and the DBB. This will provide a potential neural substrate for the observed increase in consumption of food and weight gain.

METHODS/STUDY POPULATION: (1) We will examine electrophysiological activity of the DRN and the DBB in response to optogenetic stimulation of LH fibers to these nuclei. (2) We will identify the behavioral phenotype of stimulating these same projections using optogenetic techniques. (3a) Identify the behavioral phenotype of mice possessing cre-loxP-dependent knockout (KO) of LH GABAergic activity. DRN serotonergic activity, and inhibition of DBB cholinergic activity. (3b) Using these mice, we will establish behavioral response to olanzapine in ad libitum feeding and fast-refeeding condition. (4) Using baseline and post-treatment body mass index (BMI), PANSS, and side effect profile scores from a recently completed prospective cohort study of treatment-naive schizophrenic patients receiving antipsychotics for 1 year, we will sequence multiple single nucleotide polymorphisms and explore the correlation of serotonergic, dopaminergic, and cholinergic receptor mutations with the increase in BMI and changes in PANSS score and side effect scores.

RESULTS/ANTICIPATED RESULTS: (1) Our preliminary data indicates that the LH exclusively sends GABAergic input to the DBB, and the large majority of its projections to the DRN are GABAergic. (2) We have identified that stimulating LH — > DBB projections produces intense feeding and drinking behavior, a real-time place preference for laser stimulation, and a conditioned place preference for laser stimulation. Preliminary data shows that the LH — > DRN also produces feeding behavior. (3a) Our lab has demonstrated that transgenic mice with LH-specific GABA release KO are smaller, have increased anxiety-like behaviors such a repetitive grooming and open field aversion, and have reduced feeding after fasting conditions. We expect the DRN serotonergic KO mice to have increased body weight and reduced anxiety-like behaviors. (3b) Our pilot study demonstrated that the LH GABA KO mice administered olanzapine have a greater consumption of food over 1 hour than controls (n = 7, 5, respectively; p = 0.08). DRN serotonergic KO mice and mice with inhibition of choline will have an increased baseline feeding behavior, but will not be affected by olanzapine. (4) We believe that SNPs in serotonergic receptors such as 5HT2C, and those affecting dopaminergic and cholinergic receptors, will be more common in schizophrenic patients with increased BMI than those without. Further, we believe that a reduction in the PANSS items reflecting anxiety and aversiveness will correlate with increased BMI, since we...
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.

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Innovative 3D printed intravaginal rings for contraception and HIV prevention
Rahima Benhabbour, Rima Janusziewicz, Sue J. Mecham, Roopali Shrivastava and Gayane Paravyan

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 will we 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.

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Hydrogen bonding and water accessibility changes upon expansion of PolyQ tracts in ataxin-2 and ataxin-3
Jingran Wen, Daniel Scales 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: We have established methods 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 sAC isoforms. 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 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 circuity 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.

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Investigation of sAC signaling reveals new therapeutic targets for cancer cell metabolism
Jenny Wang and Jonathan Zippin

OBJECTIVES/SPECIFIC AIMS: The soluble adenyl 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 adenyl 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 re-introduced sAC by targeting the protein to mitochondria (mito-sAC), 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 sAC 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 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 circuity 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.

In silico prediction of NS1 structure and influence 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 significantly 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