

405

Role of Glycans in Cancer Associated Fibroblast-Derived Exosome Immunoregulation

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OBJECTIVES/GOALS: To identify the role of sialoglycans in the mechanisms underlying cancer-associated fibroblast-derived exosomes (CAFEX) immuno-regulatory properties. The central hypothesis is that CAFEX manipulates the immune response to allow immunoevasion and glycomic approaches can identify the signaling factors involved. **METHODS/STUDY POPULATION:** Cancer associated fibroblasts (CAFs) were isolated from primary head and neck tumors, expanded, characterized and cryopreserved prior to experimentation and isolation of CAFEX. Sialoglycan expression was determined using lectin-specific staining of cells and bead-captured CAFEX in combination with flow cytometry analysis. Siglec expression and expression of M2-macrophage markers were also determined by flow cytometry analysis and cytokine bead arrays. Inhibition studies involved either the enzymatic removal of cell-surface sialoglycans or alternatively, a specific small molecule analog inhibitor of sialoglycan transferases. **RESULTS/ANTICIPATED RESULTS:** Both CAFs and CAFEX expressed abundant levels of cell-surface sialoglycans. CAFEX induced an M2-macrophage phenotype in primary monocytes, based on surface marker expression and cytokine secretion profiles. The induction of the M2 phenotype in macrophages was attenuated upon the removal of sialoglycans from the surface of CAFEX either by enzymatic removal or via a small molecule inhibitor. CAFEX were also able to directly bind members of the Siglec family, which are sialoglycan specific lectin receptors expressed on immune cells, including macrophages. **DISCUSSION/SIGNIFICANCE:** Collectively, these studies suggest that surface presentation of sialoglycans by CAFEX may induce an immunosuppressive phenotype in monocytes/macrophages. Consequently, this may be a novel mechanism by which cells within the tumor bed facilitate immunoevasion during tumor progression.

407

Glucose activates STAT3, promoting XRCC1 expression and increased DNA repair after exogenous challenge*

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OBJECTIVES/GOALS: Inflammation-promoting exposures, including hyperglycemia and inflammatory cytokines, are common in the development and progression of multiple cancers. Here, we determine if high glucose drives the dysregulation of XRCC1, changing BER/SSBR capacity, and increasing resistance to DNA damaging agents. **METHODS/STUDY POPULATION:** Here, we acutely exposed cell lines models with 30 mM glucose to mimic

hyperglycemic changes and monitored the gene and protein expression of STAT3 and XRCC1. We selected the osteosarcoma cell line U2OS, with high STAT3 activation before glucose challenge, and the non-tumorigenic human embryonic kidney cell line HEK293T, with low STAT3 activation before glucose challenge, to dissect the role STAT3 plays in dysregulating DNA repair. We also examined changes in STAT3 occupancy at the XRCC1 promoter following glucose challenge using chromatin immunoprecipitation (ChIP). Finally, we measured changes in the sensitivity to the alkylating agent methyl methanesulfonate (MMS) induced by the glucose challenge using cell survival and DNA strand break analysis. **RESULTS/ANTICIPATED RESULTS:** High glucose challenge increases the phosphorylation and activation of STAT3 and subsequently increases XRCC1 gene and protein content in the cell. Acute high glucose activated STAT3, driving the subsequent expression of XRCC1 in U2OS and HEK293T cells through increased STAT3 occupancy at the XRCC1 promoter. High glucose also reduced sensitivity to MMS, increasing cell survival. The most significant increase in resistance to MMS occurred in the HEK293T, which also showed the largest increase in XRCC1 protein expression following the glucose challenge. Increased survival correlated with the faster resolution of DNA strand breaks in glucose-challenged cell lines. **DISCUSSION/SIGNIFICANCE:** This work has identified a novel regulatory mechanism by which high glucose drives the expression of XRCC1 through STAT3 activation, increasing DNA repair and resistance to the DNA damaging agent MMS. These data suggest dietary choices induce sustained XRCC1 expression and may contribute to chemoresistance and poor survival outcomes in cancers.

408

In vivo calcium imaging in the medial prefrontal cortex reveals novel site of action for therapeutic effects of Neuromedin U

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OBJECTIVES/GOALS: The primary goals of this study are 1) expand our understanding of the neural circuitry influenced by the neuropeptide Neuromedin U (NMU) via its receptor Neuromedin U Receptor 2 (NMUR2), and 2) provide alternative top-down mechanisms for how NMU regulates high fat food intake and cocaine taking. **METHODS/STUDY POPULATION:** Immunohistochemistry (IHC) for NMUR2 and cell markers was performed on rat brain tissue containing the medial prefrontal cortex (mPFC). To identify the source of the presynaptic NMUR2, anterograde tracing from the paraventricular nucleus or dorsal raphe nucleus to the mPFC utilizing an AAV2- dsRed-synaptobrevin fusion protein were performed (n=3) and will be followed by IHC. Using in vivo calcium imaging technology (InScopix nVista), neuronal activity (calcium transients) was recorded from the mPFC of two awake, freely behaving rats. Each animal underwent a single session of 30 minutes baseline activity, intraperitoneal NMU administration, and 30 minutes of post-treatment activity. Activity was then processed and recorded as distinct events using the InScopix data acquisition software. **RESULTS/ANTICIPATED RESULTS:** Medial prefrontal cortex staining for NMUR2 revealed a characteristic “beads on a string” motif, consistent with presynaptic receptor expression. Furthermore, we expect the anterograde tracing experiment will show colocalization of the

dsRed-synaptobrevin fusion protein with NMUR2 on synaptic inputs into the medial prefrontal cortex. Following quantification of pre- and post-treatment events using the InScopix data acquisition software, total events during the pre- and post-treatment time periods were calculated. In these studies, both animals demonstrated a clear increase in calcium transient activity between pre- and post-treatment evaluations, suggesting that NMU administration increases the neuronal activity of neurons in the prefrontal cortex. **DISCUSSION/SIGNIFICANCE:** This research provides a new site of action for the known therapeutic effects of NMU. We demonstrate the presence of presynaptic NMUR2 in the mPFC and show that systemic administration of NMU increases mPFC neuronal activity. This illustrates NMU may act as a top-down mediator for substance use disorders and binge eating behaviors.

409

Diffusion MRI to investigate atypical corticospinal tract microstructure and motor impairments in hemiplegic cerebral palsy

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OBJECTIVES/GOALS: Hemiplegic cerebral palsy (HCP) limits the functional ability of one side of the body, but motor impairments are very heterogeneous among children with this diagnosis. The purpose of this study was to evaluate the CST using DTI and tractography analyses as it relates to quantitative measures of the severity of weakness and mirror movements in HCP. **METHODS/STUDY POPULATION:** Preliminary results include five participants with HCP (2M, 16Å±7.8 years) and six controls (2M, 12Å±3.5 years). DTI data were collected using a spin-echo echo-planar imaging sequence with diffusion weighting of $b=1000$ s/mm² in 60 different directions and 8 scans without diffusion weighting ($b=0$ s/mm²). Images were processed with steps of brain extraction, denoising, motion and eddy current correction, and fit with tensors to generate maps of diffusivity metrics. Anatomical landmarks were used to guide probabilistic tractography of the CST for analyses in both the lesioned and non-lesioned hemispheres. To quantify grasp weakness and mirroring severity, participants completed a bilateral assessment of grip strength using handheld force measurement devices and custom MATLAB data acquisition software. **RESULTS/ANTICIPATED RESULTS:** DTI is a feasible method to evaluate CST microstructure in HCP and typically developing pediatric participants. Spearman correlation analyses, using age and sex as covariates, revealed that for the lesioned hemisphere CST, there were significant positive correlations between grasp weakness severity and mean diffusivity (MD) ($\bar{r}=0.66$, $p=0.038$) and between grasp weakness severity and axial diffusivity (AD) ($\bar{r}=0.68$, $p=0.030$). There was not a significant correlation between grasp weakness severity and fractional anisotropy (FA) ($\bar{r}=-0.47$, $p=0.166$). For the non-lesioned hemisphere CST, there was a significant positive correlation between mirroring severity and radial diffusivity (RD) ($\bar{r}=0.70$, $p=0.023$). There was not a significant correlation between mirror movement severity and FA ($\bar{r}=-0.41$, $p=0.2361$). **DISCUSSION/SIGNIFICANCE:** The correlations demonstrated here show a potential relationship between CST microstructure and the severity of hand impairments in HCP. While these relationships between CST diffusivity properties and hand function are preliminary, they provide the first steps to better understand underlying neural mechanisms for motor impairments in HCP.

410

Mild Maternal Undernutrition Results in a Premature Neonatal Leptin Surge and Resistance in Male Offspring to a High Fat Diet[†]

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OBJECTIVES/GOALS: Maternal undernutrition, a form of malnutrition, can alter neonatal leptin signaling and result in metabolic dysfunction in adulthood. We developed a mild undernutrition model to relate more to society's nutritional challenges and to test the hypothesis that a shift in the neonatal leptin surge would result in sex-specific metabolic changes. **METHODS/STUDY POPULATION:** We studied pups from undernourished dams which were calorically restricted by 20% (CR20) from embryonic day 15 until postnatal day (PND) 21. We tested 216 offspring from 11 Fed dams and 13 undernourished dams (CR20), detecting a leptin surge in control fed progeny at PND11. At 3 months of age, offspring from 3 dams per maternal nutrient status were either exposed to a 45% high fat diet (HFD) or control diet (10% fat) for 16 weeks. Anterior pituitary hormones were analyzed in the pituitary and serum of neonates and adults. To determine the mechanism of the phenotype observed in male adult offspring on the HFD, single cell RNA sequencing was used to analyze the pituitary, fat and liver. **RESULTS/ANTICIPATED RESULTS:** Offspring of CR20 dams had an early leptin surge peaking at PND8 and GH levels at PND1 were higher in CR20 progeny. Weights of both male and female CR20 offspring were lower and body lengths were shorter than controls. As adults, Fed mice from both sexes had increased weight gain with HFD. However, although CR20 females gained weight on the HFD, male progeny from CR20 dams did not gain weight on the HFD and appeared protected from impact. We found sex-specific changes in pituitary Gh, Ghrhr, and Ghnr mRNA levels. Single cell RNA sequencing of pituitary, fat and liver of male offspring showed significant regulation of transcripts in fat of male offspring from Fed dams that was not found in CR20 males when compared to control fed mice. **DISCUSSION/SIGNIFICANCE:** Mild undernutrition causes a prematurely high leptin surge and sex-specific growth responses to a HFD, including resistance to a HFD in underfed males. Transcript analysis in fat of males resistant to HFD induced obesity may reveal mechanisms that provide protection against HFD induced weight gain.

411

Essential role for the neurodevelopmental disorder-linked gene, MEF2C, in inhibitory neuron function and neurotypical behaviors*

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OBJECTIVES/GOALS: The MEF2 family of transcription factors regulate gene expression controlling cell differentiation and synapse development. Mutations or deletions in the MEF2C gene cause a neurodevelopmental disorder that includes symptoms of autism spectrum disorder. In this study, we aim to study the role of MEF2C in GABAergic populations using an animal model. **METHODS/**