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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.

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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.

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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

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