represented on a signal bar display and represented the average cingulate activation during the trial. Unlike many rtfMRI studies, the purpose here was not for participants to interact with the neurofeedback directly. Rather, a feedback summary was shown to participants after each MEMORY and STRATEGY trial as an index of how brain activity changed in response to negative memories/worries and therapeutic strategies. Our goal was not for participants to learn to self-regulate the cingulate cortex, but rather to provide participants with a metacognitive demonstration of strategy efficacy. Participants were given detailed instructions regarding the task design, the role of the cingulate cortex in depression, as well as the hypothesized direction of activation during the MEMORY and STRATEGY phases to help them interpret the neurofeedback. RESULTS/ANTICIPATED RESULTS: Results revealed that "stronger neurofeedback" (defined as the difference between STRATEGY vs. MEMORY trials) correlated with self-reported strategy efficacy ratings immediately following the scan session ($p < 0.05$). More importantly, stronger neurofeedback predicted both self-reported strategy efficacy and frequency of use 1 month following the MRI session ($p < 0.05$). Importantly, this relationship was specific to only those strategies used inside the scanner; and no such relationship was observed at baseline. Neuroimaging results revealed that during the MEMORY phase, activation within inferior frontal gyrus and supramarginal gyrus correlated with baseline BDI score (whole brain, cluster corrected with FSL Flame 1 to $p < 0.05$). During the STRATEGY phase, the periaqueductal gray nucleus, insula, and temporal pole predicted self-reported frequency of strategy use 1 month post-scan session (whole brain, cluster corrected with FSL Flame 1 to $p < 0.05$). DISCUSSION/SIGNIFICANCE OF IMPACT: We believe this study holds promise to provide a powerful demonstration for individuals that strategies used to cope with negative moods can produce significant changes in their brain.

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Vesicular secretion of suppressor of cytokine signaling 3 by alveolar macrophages is dysregulated in NSCLC patients and its provision inhibits epithelial cell transformation and tumor cell function

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OBJECTIVES/SPECIFIC AIMS: Insufficient endogenous expression of suppressor of cytokine signaling 3 (SOCS3) with subsequent over-activation of its target, the transcription factor STAT3, has been associated with tumorigenesis and cancer development in the lung and other organs. We have observed that a "backup" source of SOCS3 in the lung, namely that secreted in microvesicles (MVs) by alveolar macrophages, is reduced in the bronchoalveolar lavage fluid (BALF) of KRAS mutant mice harboring lung tumors. Here we sought to evaluate levels of SOCS3 in BALF of a cohort of non-small cell lung cancer (NSCLC) patients and to test the effects of vesicular SOCS3 administration on tumor cell transformation and function as potential therapeutic strategy. METHODS/STUDY POPULATION: In total, 22 BALF samples were obtained from healthy volunteers (n = 11) as well as patients undergoing diagnostic bronchoscopies for suspected lung cancer (n = 11). SOCS3 levels in the BALF were determined by ELISA after brief sonication to disrupt vesicles. In vitro experiments utilized the human adenocarcinoma cell line (A549) or human G12V mutant KRAS-expressing rat lung epithelial cells (RLE-G12V). Proliferation, Fas ligand (FasL)-induced apoptosis, and chemical transformation with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) or cigarette smoke extract (CSE) were assessed by CyQuant assay, annexin V staining, and soft agar assay, respectively. For SOCS3 rescue, epithelial cells were treated with natural alveolar macrophages-derived MVs (isolated via ultracentrifugation) or synthetic unilamellar liposomes containing human recombinant SOCS3 for at least 1 hour before assay. RESULTS/ANTICIPATED RESULTS: SOCS3 levels were significantly reduced in BALF samples of patients determined to have NSCLC as compared with healthy volunteers (186.6 ± 26.74 vs. 395.6 ± 74.31 pg/mL, $p = 0.015$, n = 11). Addition of exogenous SOCS3-containing liposomes had the capacity to significantly inhibit MNNG and cigarette smoke extract-induced transformation and colony formation in soft agar. Exogenous SOCS3 provided in liposomes or in natural MVs significantly induced apoptosis (both in the presence and absence of FasL) and inhibited basal proliferation of A549 cells. DISCUSSION/SIGNIFICANCE OF IMPACT: These data identified a novel dysregulation of immune surveillance in the form of decreased SOCS3 secretion in the tumor-bearing lung that may contribute to tumorigenesis via sustained STAT3 activation. Future studies will focus on the mechanism underlying this defect and whether rescuing SOCS3 secretion can inhibit cancer progression in vivo.

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What genes are involved in the brain food reward circuitry: Findings from a large candidate gene analysis

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OBJECTIVES/SPECIFIC AIMS: The food reward circuitry regulates hedonic eating especially in relation to palatable hypercaloric foods, which can lead to chronic overeating and consequent overweight and obesity. Evidence supports that there is considerable overlap within the brain reward circuitry between palatable hypercaloric food intake and substance addiction. The goal of this study was to identify associations between addiction-related genes and body mass index. We hypothesized that addiction-related genes potentially participate in the food reward circuitry if they are associated with obesity traits. METHODS/STUDY POPULATION: A secondary analysis was conducted with 1093 African American adolescents and young adults from the New Mother's Study. Anthropometric, genetic, demographic and lifestyle measurements were available at the 18-year follow-up assessments. A total of 1350 single nucleotide polymorphisms mapped to 127 addiction-related genes were assessed. A total of 186 ancestry informative markers were used to adjust for population stratification. Generalized estimating equation models were used to identify genetic associations, including additive, dominant, and recessive models, and control for correlations within families. RESULTS/ANTICIPATED RESULTS: The participants ranged from 15 to 23 years of age. Of them, 42.7% were overweight or obese. Significant associations with body mass index were identified for 13 single nucleotide polymorphisms mapped to 11 addiction-related genes, including LEP (p 0.027–<0.001). Most of these genes are involved in dopaminergic, opioidergic, serotonergic pathways, and stress. DISCUSSION/SIGNIFICANCE OF IMPACT: Our results support the role of dopaminergic and opioidergic pathways in the food reward circuitry, and suggest a potential involvement of serotonergic pathways and genes related to stress in the food reward circuitry. Further investigation of the identified genes will facilitate delineation and understanding of the brain food reward system and its relationship with obesity.

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Validation of a novel PD-L1 assay for bladder cancer circulating tumor cells

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OBJECTIVES/SPECIFIC AIMS: Bladder cancer patients being considered for immune checkpoint blockade are often judged on immunohistochemical staining for the checkpoint target protein PD-L1 in the original surgery or biopsy sample. However, sampling error or the clinical evolution of most patients' cancer can render the original PD-L1 assessment no longer accurate. In contrast, circulating tumor cells (CTCs) allow serial noninvasive sampling of the current tumor status throughout a patient's clinical course including those with the highest metastatic potential. We therefore sought to develop a method for quantifying PD-L1 expression in CTCs towards addressing inherent limitations of current UC management. METHODS/STUDY POPULATION: This work utilizes both cancer cell lines as well as patient samples. Positive and negative control cancer cell lines were assessed via "industry standard" antibodies for PD-L1 expression via Western blots and immunofluorescence, and a threshold-based method was developed for reliable quantification. PDL-1 expression was additionally verified via interferon-mediated up-regulation. CTCs isolated from bladder cancer patient samples via a density centrifugation method were then assessed for PD-L1 via the same antibodies. RESULTS/ANTICIPATED RESULTS: We will show preliminary preclinical and clinical data that validates the sensitivity and specificity of our assay. A case study will be presented that illustrate the potential useful of the novel approach we describe and which should be complementary to current clinical practices. In a patient with metastatic bladder cancer, this method effectively detected the PD-L1 expression in CTCs taken at a time coincident to when the patient derived an

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