metastatic in chronic wounds, burns, and in patients with recessive dystrophic epidermolysis bullosa (RDEB). METHODS/STUDY POPULATION: We used CRISPR/Cas9 gene-editing to knock out HMGB1 in a keratinocyte line, p16INK4a-negative keratinocytes immortalized by ectopic hTERT expression (N/TERT-2G [46, XY]). Following gene editing, clonal keratinocyte populations were screened for knockout by PCR followed by TIDE analysis (Tracking of Indels by Decomposition) to identify indels that would result in a frameshift mutation. Total cell lysates for each clonal population were analyzed by immunoblot and immunofluorescence for HMGB1 protein. These cells will be used to assay for DNA damage sensitivity in the presence of genotoxic agents (etoposide, ultraviolet radiation, γ-irradiation). A lentiviral vector will then be used to express mutant forms of HMGB1 that localize to the nucleus (C23/45S) or cytoplasm (C106S) and DNA damage assays repeated. RESULTS/ANTICIPATED RESULTS: We have confirmed by sequencing, immunoblot, and immunofluorescence that HMGB1 is knocked out in a clonal population of N/TERT-2G human keratinocyte cells. We anticipate that cells with a complete absence of HMGB1 will have high sensitivity to DNA damaging agents, but little change in inflammatory signaling. We also expect that cells expressing mutant HMGB1 that localizes exclusively to the cytoplasm will demonstrate an increased sensitivity to DNA damage relative to wild-type controls, while mutant HMGB1 that localizes exclusively to the nucleus will be protected from DNA damage caused by exposure to genotoxic agents. DISCUSSION/SIGNIFICANCE: HMGB1 is a nuclear protein and damage associated molecular pattern that is elevated systemically in patients with RDEB, many of whom will go on to develop metastatic squamous cell carcinoma. The experiments described here investigate whether HMGB1 plays a mechanistic role in skin carcinogenesis via regulation of the DNA damage response.

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Identification of potential targets for immunotherapy in a cynomolgus macaque model of Ebola virus disease

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OBJECTIVES/GOALS: Ebola virus infection causes severe disease and liver injury in humans. Macrophages contribute to inflammatory signaling and are prevalent in the liver. We assessed the activation status, including therapeutic target expression, of hepatic macrophages. METHODS/STUDY POPULATION: We compared formalin-fixed, paraffin-embedded liver tissue from terminal Ebola virus-infected and uninfected control cynomolgus macaques, a gold-standard model for human disease. We characterized region-specific protein and whole transcriptome expression in these tissues using GeoMx Digital Spatial Profiling. Macrophage (CD68+) and leukocyte (CD45+) accumulation in liver tissue was quantified by immunofluorescence image analysis using digital pathology software. RESULTS/ANTICIPATED RESULTS: Macrophage-specific (CD68+) regions in the liver of Ebola virus-infected macaques demonstrated a shift towards an inflammatory gene expression profile, as compared to those from healthy control tissue. These regions showed differential expression of monocyte/macrophage differentiation, antigen presentation, and T cell activation gene sets, which were associated with decreased MHC-II allele expression. Moreover, macrophage-specific regions in the infected macaques showed enriched expression of genes or proteins associated with known immunomodulatory therapeutics, including S100A9, IDO1, and CTLA-4. DISCUSSION/SIGNIFICANCE: These data demonstrate that hepatic macrophages express an inflammatory phenotype, that their ability to present antigens to the adaptive immune system may be impaired, and that they express therapeutically targetable markers for immunomodulation of these cells during Ebola virus infection.

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Identification of Psychosis Risk Biomarkers in 22q11DS for future translational studies

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OBJECTIVES/GOALS: 22q.11 deletion syndrome (22q11DS) is a genomic syndrome that elevates risk for psychosis >20-fold. We used a battery of cognitive and psychophysiological psychosis-risk biomarkers in 22q11DS patients and healthy subjects in order to identify biomarkers of psychosis in 22q11DS that could be used as translational targets in intervention studies. METHODS/STUDY POPULATION: We recruited 15 22q11DS individuals (Mean age=30, M/F=9/6) and 19 healthy controls (HCs; Mean age=34, M/F=5/14). Each individual completed the MATRICS Consensus Cognitive Battery (MCCB), the Wechsler Abbreviated Scale of Intelligence, Second edition (WASI-II) Verbal IQ subtests, and the computerized Wisconsin Card Sorting Task (WCST). To examine auditory EEG responses, each participant completed the 'Double-Deviant' target detection paradigm, which presents a pseudorandom sequence of frequent standard tones and infrequent deviant tones. Mismatch negativity (MMN) metrics were generated from this assessment. Welch's t-tests were completed for neurocognitive variables. One-Way ANOVAs were completed to examine EEG results, with sex entered as a separate factor and age entered as a covariate. RESULTS/ANTICIPATED RESULTS: Significant group differences were found in 8 of the 9 neurocognitive measurements (FDR-adjusted p's< 0.02, average Cohen's d=1.62, average observed power= 0.91) indicating widespread cognitive deficits in 22q11DS subjects across multiple domains. The Double-Deviant MMN ERP response was significantly smaller in absolute magnitude in the 22q11DS group (FDR-adjusted p=0.048, Cohen's d= -0.864, observed power= 0.58). The MMN ERPs for the frequency and duration deviants were not significantly different (FDR-adjusted p's> 0.33). No group by sex interactions were observed in any of the measures. Neurocognitive variables were associated with psychosis positive, negative, general, and disorganized symptom scales. DISCUSSION/SIGNIFICANCE: Our results identify potential psychosis-risk biomarkers in 22q11DS. If replicated, these biomarkers could provide important translational targets for future clinical trials for individuals with 22q11DS and other individuals at-risk for psychosis syndromes.

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