methods to define TCR specificity after melanoma patients treated with TCR engineered T-cells suffered from fatal cardiovascular toxicity arising from the unpredicted recognition of a muscle-specific peptide. METHODS/STUDY POPULATION: To address this drawback to T-cell-based immunotherapies, we developed a novel protein engineering approach to define the peptide specificity of a given TCR. Here, directed evolution in a yeast display system produced a large scale peptide library, where recognition by the melanoma reactive DMF5 TCR acted as the guiding selective pressure. After this technique identified a panel of putative cross reactive peptides, sequence analysis and computational modeling followed by kinetic binding experiments and structural analysis determined the DMF5 TCR recognizes 2 distinct classes of peptides through chemically distinct mechanisms. RESULTS/ANTICIPATED RESULTS: This information led to the rational, structure-based design of additional cross reactive peptides and introduced a unique approach to screen the human proteome and identify the TCR targets which triggered undesired autoimmunity when this molecule was used in clinical trials. DISCUSSION/SIGNIFICANCE OF IMPACT: The distinct chemical nature of the 2 peptide classes suggest TCRs are more cross reactive than previously thought, presenting an obstacle to cell-based immunotherapy. Defining the peptide specificity of TCRs is of high interest to the immunology community, and will lead to improved approaches to designing engineered TCRs for cell therapy.

2074

Comparing the properties of human umbilical cord-derived mesenchymal stromal cells from preterm Versus full-term infants

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OBJECTIVES/SPECIFIC AIMS: To compare functional differences in WJ-MSCs-derived from term Versus preterm infants. METHODS/STUDY POPULATION: WJ-MSCs were enzymatically digested from umbilical cord tissue from Term (gestational age ≥37 wk, n = 4) and Preterm (gestational age ≤32 wk, n = 5) neonates. Cells were characterized by (1) surface antigen markers using flow cytometry, (2) ability to differentiate into adipogenic, chondrogenic, and osteogenic lineages following in vitro stimulation, (3) colony forming unit efficiency, (4) proliferation rates, and (5) cell motility assay. RESULTS/ANTICIPATED RESULTS: WJ-MSCs were successfully isolated from both Preterm and Term groups. Cells adhered to plastic and displayed characteristic spindle-shaped morphology when cultured under standard conditions. WJ-MSCs from both groups expressed surface antigen markers CD73, CD90, and CD146 (≥90%) and did not express hematopoietic markers HLA-DR, CD79, or CD117 (<5%). Preterm and Term cells were capable of differentiating into osteogenic, chondrogenic, and adipogenic lineages. There were no significant differences between the groups when evaluated by colony forming efficiency, proliferation rates, or cell motility. DISCUSSION/SIGNIFICANCE OF IMPACT: These preliminary findings suggest that WJ-MSCs derived from full-term or preterm neonates have similar functional characteristics. Future studies will focus on the regenerative potential of WJ-MSCs from preterm and term infants following changes in the microenvironment (eg, oxygen tension, inflammatory stimulation).

2078

NGF and TNF-α contribute to oral cancer pain by regulating pro-inflammatory cytokines

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OBJECTIVES/SPECIFIC AIMS: We hypothesize that both NGF and TNF-α contribute to oral cancer pain by upregulating pro-nociceptive inflammatory cytokines. METHODS/STUDY POPULATION: In total, 48 oral cancer patients were evaluated and their pain scores were measured using a validated oral cancer pain questionnaire. Presence of perineural invasion (PNI) was identified from patients' pathology reports. We utilized The NIH Cancer Genome Atlas (TCGA) Head and Neck Cancer cohort to investigate the association between pain and genes related to NGF, TNF-α, and their receptors (TRKA, P75, TNF-α receptor 1, and TNF-α receptor 2) in oral cancer samples by employing PNI as a surrogate for pain. Demographic characteristics, clinical characteristics, and genes were analyzed using logistic regression models. A xenograft cancer pain model was created by inoculating human oral cancer cells (HSC-3) into the mouse hind paw. Mice (n = 6 per group) were treated with anti-NGF alone, anti-TNF-α alone, a combination of anti-NGF and anti-TNF-α, or PBS (vehicle control). Nociceptive behaviors were measured using an electronic paw withdrawal assay. Paw volume was measured using a plethysmometer. Cytokines in the paw tissues were measured using a multiplex assay kit with 28 cytokines. RESULTS/ANTICIPATED RESULTS: Oral cancer patients with PNI report significantly more pain compared with patients without PNI in our patient cohort ($p < 0.05$). From analysis of TCGA data, we found that PNI is significantly associated with lymphovascular invasion, pathological nodal invasion, and pathological tumor staging (all $p < 0.05$). In adjusted models, we observed that the NGF receptor p75NTR (NGFR) and the TNF-α receptor 1 (TNFRSF1A) were associated with PNI (both $p < 0.05$) and significantly correlated to each other ($r = 0.25$, $p < 0.001$). High levels of TNF-α were present in HSC-3 cell lines and the mouse xenograft cancers. In mice with cancer pain, combined treatment with anti-NGF and anti-TNF-α together provided more effective pain control compared with either anti-NGF or anti-TNF-α treatment alone ($p < 0.05$). We found significantly increased levels of MIP3a, IL-1b, IL-2, IL-4, IL-28b, IL-23, IL17a, IL-31, and IL-33 in cancer mice compared with normal mice (all $p < 0.05$). The combination therapy significantly reduced cytokines MIP3a, IL-1b, IL-4, IL-28b, IL-31, and IL-33 (all $p < 0.05$). DISCUSSION/SIGNIFICANCE OF IMPACT: We show that targeting both NGF and TNF-α provides more effective pain relief in an oral cancer model. These results suggest that therapeutic strategies aimed at both pathways could yield improved pain management for oral cancer patients.

2080

Therapeutic potential of mesenchymal stromal cells for hypoxic ischemic encephalopathy: A systematic review of preclinical studies

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OBJECTIVES/SPECIFIC AIMS: To assess the efficacy of exogenous administration of MSCs in animal models of HIE. METHODS/STUDY POPULATION: Adhering to the Systematic Review Protocol for Animal Intervention Studies, a systematic search of English articles was performed using MEDLINE, Web of Science, CINAHL, and Google Scholar. Search term items included mesenchymal stem/stromal cell, hypoxic ischemic encephalopathy, asphyxia, cerebral ischemia, and neonatology. We selected randomized and nonrandomized studies that examined in vivo neonatal models of induced HIE. We excluded studies that combined MSCs with an adjunct therapy. Data were collected on study specifics, MSC characteristics, and outcome measurements. The primary outcome was efficacy of MSC treatment, assessed by functional neurologic measures (cognitive, motor, sensory). Risk of bias was assessed using the SYRCLE's risk of bias tool and we used the ARRIVE guidelines to describe the quality of study reporting. RESULTS/ANTICIPATED RESULTS: A total of 17 preclinical publications focusing on MSC therapy for HIE met our inclusion criteria. Fifteen of the studies (88%) induced HIE in rodents by ligating the common carotid artery followed by a period of hypoxic exposure. Nine (53%) studies derived their MSCs from rodent bone marrow, whereas the other investigators provided xenografts from human bone marrow or umbilical cord-derived MSCs. Range of MSC dose was between 0.25 and 3.5 × 106 cells with 71% of the experiments transplanting the MSCs intranasally or intracerebral. Three studies (18%) administered multiple doses. The cylinder rearing test was the most common (73%) sensorimotor functional outcome performed in the first month following the establishment of HIE. All studies demonstrated a reduction in asymmetrical paw preference after receiving MSC therapy. Lesional size was assessed, using neuroimaging or histologic evaluations, and the majority of studies showed a decreased insult following MSC therapy. DISCUSSION/SIGNIFICANCE OF IMPACT: MSC treatment demonstrates improved functional and structural outcomes that are encouraging for future translational studies.

2081

Phenotypic characterization of the CD4+ T-cell response during anti-CTLA4 therapy with ipilimumab in melanoma patients

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OBJECTIVES/SPECIFIC AIMS: To characterize the CD4+ T-cell response during CTLA-4 blockade immunotherapy with ipilimumab in patients with metastatic melanoma by correlating cytokine profiles with phenotypic changes in the intratumoral lymphocyte compartment of tumor biopsies obtained before and after treatment. METHODS/STUDY POPULATION: Peripheral

https://doi.org/10.1017/cts.2017.198 Published online by Cambridge University Press