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Building A Better CAR: Improving CAR-T Trafficking in Cancer Therapy†

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OBJECTIVES/GOALS: #NAME? METHODS/STUDY POPULATION: Cell culture & protein identification: human T cells were purified from healthy blood, then activated & cultured for 5d. CAR-T cells were collected from infusion bags of cancer patients undergoing CAR-T. Silver staining of naive & activated healthy T-cell lysates was compared; B-II spectrin was upregulated and confirmed by Western blot. Migration assays: naive & activated T-cells were imaged during migration on ICAM-1 and ICAM-1 + CXCL12 coated plates. T-cells were transfected with BII-spectrin cDNA & the chemokine dependence of migration was compared with controls. In-vivo studies: in a melanoma mouse model, BII-spectrin transfected or control T-cells were injected; tumors were followed with serial imaging. Human patient records were examined to correlate endogenous BII-spectrin levels and CAR-T response. RESULTS/ANTICIPATED RESULTS: Activated T-cells downregulate the cytoskeletal protein B-II spectrin compared to naive cells, leading to chemokine-independent migration in in vitro assays and off-target trafficking when CAR-T cells are given in vivo. Restoration of B-II spectrin levels via transfection restores chemokine-dependence of activated T-cells. In a mouse melanoma model, control mice injected with standard activated T-cells showed fewer cells in the tumor site and more cells in the off-target organs (spleen, lungs) when compared to mice injected with B-II spectrin transfected cells. Furthermore, among 3 human patients undergoing CAR-T therapy, those with higher endogenous B-II spectrin levels experienced fewer side-effects, measured by the neurotoxicity and cytokine release syndrome grades. DISCUSSION/SIGNIFICANCE: A major hurdle to widespread CAR-T therapy for cancer is significant, often fatal side-effects. Our work shows that the protein B-II spectrin is downregulated during CAR-T production, and that restoring B-II spectrin levels decreases side-effects while increasing tumor clearance–hopefully translating to better CAR-T regimens for the future.

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Genetic Compensation as a mechanism underlying patients with Rare ALS

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OBJECTIVES/GOALS: Rare mutations in CHCHD10 gene are found in 1% of patients with familial Amyotrophic lateral sclerosis (ALS). The overall goal of this study is to utilize induced pluripotent stem cells (iPSCs) as an in vitro model organism for rare ALS variants to evaluate the mechanism of transcription adaptation of CHCHD10/2 as a potential therapeutic. METHODS/STUDY POPULATION: Point mutations on normal iPSCs was performed via Donorguide CRISPR/Cas9. The single stranded RNA/DNA donors contain genetic alterations of CHCHD10: Pro12Ser, Arg15Leu, Pro23Leu, Pro34Ser, Ser59Leu, Gly66Val, Pro80Leu, Tyr92Cys and Gln102His. Ribonucleoprotein electroporation was used to transfect iPSCs and DNA sequencing was used to validate gene editing. To validate transcriptional adaption, changes in levels of protein and gene expression were measured via immunoblot and quantification of CHCHD10 and CHCHCD2 was performed from whole cells lysates of the edited iPSCs. RESULTS/ANTICIPATED RESULTS: We anticipate that CHCHD2 transcriptional adaptation can functionally compensate for the locus loss of function of CHCHD10. This mechanism of transcriptional adaptation may contribute to an explanation for variation in clinical manifestations of patient phenotypes. DISCUSSION/SIGNIFICANCE: This study supplies further evidence for genetic modification as a treatment option for diseases with point mutation causal or enabling mechanisms, including some variants of ALS. Future work will explore the gene-correction from an ALS patient with a known CHCHD10-R15L variant.

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Impact of CDK4/6 inhibitor-induced cellular senescence on stromal responses to breast cancer

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OBJECTIVES/GOALS: The objective of my study is to test my overall hypothesis that CDK4/6 inhibitor-induced cellular senescence has long-lasting consequences on the tumor microenvironment that affect tumor growth and disease progression. METHODS/STUDY POPULATION: There is a lack of mechanistic understanding of the crosstalk of drug effects on different cellular pathways. One less-studied consequence of CDK4/6 inhibitors (CDKI) is their ability to induce cellular senescence in the non-malignant host cells ("stroma"), which causes secretion of a plethora of proteins that have lasting effects on the tumor microenvironment. Stromal cells are known to be able to enter senescence and promote a state of chronic inflammation that contributes to disease progression. I propose to study the stromal contribution to the tumor microenvironment and how this affects tumor growth and disease progression by using genetically engineered mouse models of breast cancer. RESULTS/ANTICIPATED RESULTS: We have found that palbociclib, a CDKI, inhibited growth and induced senescence of breast cancer cell lines xenografted into mice, and that while most on-target effects of palbociclib were reversed after treatment cessation, there was long-lasting down-regulation of interferon-Î³ and inflammatory response signaling pathways in the mouse host stroma that persisted after cessation of palbociclib treatment. In syngeneic models, we have found that palbociclib treatment influenced migration of T cells into CDKI-resistant tumors. DISCUSSION/SIGNIFICANCE: These studies will shed light on how senescence-inducing anticancer therapies, such as CDKI, affect tumor growth and disease progression. My studies will help elucidate new therapeutic avenues in combination with CDKI to combat therapeutic resistance in advanced breast cancer.

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