

absence of GR ligands, GR is transcriptionally activated via p38-MAPK-dependent phosphorylation of Ser134 upon exposure of TNBC cells to TME-derived agents (TGF β , HGF). The ligand-independent pS134-GR transcriptome primarily encompasses gene sets associated with TNBC cell survival and migration/invasion. Accordingly, pS134-GR was essential for TGF β -induced TNBC cell migration, anchorage-independent growth in soft-agar, and tumorsphere formation, an *in vitro* readout of breast cancer stemness properties. Finally, a 24-gene pSer134-GR-dependent signature induced by TGF β 1 predicts shortened survival in breast cancer. We expect to find similar results using an in-house tissue microarray. **DISCUSSION/SIGNIFICANCE OF IMPACT:** Phospho-S134-GR is a critical downstream mediator of p38 MAPK signaling and TNBC migration, survival, and stemness properties. Our studies define GR as a required effector of TGF β 1 signaling and nominate pS134-GR as a biomarker of elevated risk of breast cancer dissemination.

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Identification of small molecules that facilitate the efficient differentiation of stem cell derived β -cells*

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OBJECTIVES/GOALS: In this study, we established a high-throughput chemical screening platform to identify small molecules that facilitates efficient differentiation of stem cells derived β (SC- β) cells. Using this platform, we identified several compounds that potentially increase the differentiation efficiency. **METHODS/STUDY POPULATION:** Differentiation of human embryonic stem cells (HUES8) into SC- β was carried out using previously published protocols in a 3D cell suspension. Single cells were replated in Matrigel-coated well plates at the start of different stages depending on experiments. Differentiation medium supplemented with small molecules at a final concentration of 2 M and 0.2 M was used throughout the stage. All the cells were then fixed and permeabilized. Immunocytochemical staining was performed. Images of each well were taken and analyzed. Numbers of the total cell, insulin-positive cell, NKX6.1-positive cell, and co-positive cell were recorded. Candidate compounds were validated using flow cytometry or ICC. **RESULTS/ANTICIPATED RESULTS:** We identified several hit compounds that significantly increase the NKX6.1 positive cell percentage compared to the DMSO-treated controls when treated at the PP1 cell stage. Follow up assays demonstrated that at least one of these putative hits reproducibly increased NKX6.1 expression. In addition, we identified other compounds that significantly increase the insulin and NKX6.1 copositive SC- β cell population when treated at the later PP2 cell stage during the differentiation. We expect a dosage-dependent response when the candidate hits are validated using more accurate assays. **DISCUSSION/SIGNIFICANCE OF IMPACT:** We established a high-throughput screening platform to identify small molecules that increase the efficiency of SC- β direct differentiation. Successful generation of SC- β allows cell replacement therapy in diabetes patients, and a better understanding of pancreatic biology and development.

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Impaired Natural Killer Cell Function May Be Associated with Cancer-related Fatigue

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OBJECTIVES/GOALS: During and after cancer treatment, cancer-related fatigue (CRF) is a debilitating symptom reported by up to 80% of cancer patients. Our understanding of the pathology underlying CRF is limited. Preliminary RNA sequencing data suggest that increased levels of KIR3DL1, the natural killer cell (NK) immunoglobulin-like receptor 3DL1a, may be associated with CRF. **METHODS/STUDY POPULATION:** Fatigue was measured using the Functional Assessment of Chronic Illness Therapy-Fatigue (FACT-F). Functional validation of the NK cell finding was performed from whole blood obtained from fatigued and non-fatigued subjects. NK cells were isolated from freshly collected whole blood using a human NK cell isolation kit based on CD56 microbead positive selection. NK cell function was assessed using the NK cell direct cytotoxicity assay. Briefly, isolated NK cells were co-cultured in a 2:1 ratio with calcein AM-labelled K562 cells, which are NK cell-sensitive due to the very low MHCI expression. NK cell-mediated cytotoxicity was assessed with Cytation 1 Cell Imaging Multi-Mode reader. Flow cytometric protocols were used to examine NK subset differences between the fatigued and non-fatigued groups. **RESULTS/ANTICIPATED RESULTS:** NK cells isolated from the fatigued group exhibited decreased cytotoxicity at 12.28% compared to NK cells isolated from non-fatigued controls at a mean of 40.6% cytotoxicity. Flow cytometry analysis revealed a decrease in the CD56^{dim} CD16^{bright} population in the fatigued group (87.1% of CD56+CD4- cells) compared to the control (91.4% of CD56+CD4- cells). Furthermore, there was a decrease in NKG2A expression in mature NK cells (CD56^{dim} CD16^{bright}) isolated from the fatigued group compared to the non-fatigued group. **DISCUSSION/SIGNIFICANCE OF IMPACT:** Results from the pilot study suggest that there was a decrease in NK cell cytotoxicity in the fatigued group. In addition, there may be a shift in NK cell subpopulations associated with fatigue. Findings from this pilot study suggest that impaired NK cell function may be associated with CRF pathogenesis.

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Inflammation partially mediates fatigue-like behavior in mice

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OBJECTIVES/GOALS: Fatigue is a distressing side effect of cancer and its treatment. It is a subjective symptom that can include mental, physical, emotional, and motivational components. We sought to determine whether preventing inflammation affects fatigue-like behavior in a mouse model of radiation therapy. **METHODS/STUDY POPULATION:** C57BL/6 mice received three consecutive 8 gray doses of daily peripheral irradiation. We used voluntary wheel running activity to measure fatigue-like behavior before and after this period. Minocycline, an antibiotic with anti-inflammatory effects, was administered beginning a week before irradiation and