lymphomas in vitro and in vivo in immunodeficient NSG mouse models.
Conclusions Collectively, these data identify promising combinations of AFM13 with cytokine-activated adult blood or cord blood NK cells against CD30+ hematologic malignancies, warranting clinical trials with these novel combinations.

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532 SOCS3 DEFICIENCY BLOCKED AUTOPHAGY-DEPENDENT MYELOID DIFFERENTIATION OF EARLY-STAGE MYELOID-DERIVED SUPPRESSOR CELLS VIA THE MIR-155/C/EBPß/WNT AXIS

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Background Early-stage myeloid-derived suppressor cells (eMDSCs) are a newly defined subset of myeloid-derived suppressor cells (MDSCs) that accumulate densely in tumors and potently promote tumor growth and metastasis by suppressing antitumor immune responses in vitro and in vivo. We previously identified a subset of eMDSCs in human breast cancer with a characteristic phenotype of Lin-HLA-DR-CD33+. We also found that SOCS3 deficiency and sustained activation of the JAK/STAT signaling pathway are critical molecular events coordinating the differentiation of eMDSCs, although the distinct molecular regulation has not been fully elucidated.
Methods Herein, we genetically constructed conditional SOCS3 knockout mice with SOCS3 deficiency specifically in the myeloid linage (SOCS3MyeKO). We analyzed the number of eMDSCs in SOCS3MyeKO mice (eMDSCsSOCS3KO). To explore which pathways participated in dysfunctional eMDSC differentiation, we performed whole-genome RNA sequencing and miRNA microarray on CD11b+Gr-1+ cells, eMDSCsfl/fl and eMDSCsSOCS3KO to screen the potential regulatory ceRNA network in eMDSCsSOCS3KO. CD11b+Gr-1+ cells isolated from SOCS3fl/fl mouse spleens were used as mature myeloid cell controls. Furthermore, we applied a specific miR-155 antagonist and the autophagy agonist rapamycin to suppress tumor growth and eMDSC infiltration.
Results The transcriptome results and corresponding intervention experiment revealed that the differentiation block in eMDSCsSOCS3KO was caused by SOCS3 deficiency-mediated limited autophagy activation in an AMPK-independent manner. The results of miRNA microarray and RNA sequencing demonstrated that miR-155 overexpression and Wnt/ß-catenin pathway activation were involved in the SOCS3 knockout-mediated myeloid differentiation block and autophagy repression. Further experiments revealed that miR-155 was induced by activation of the STAT3/NK-?B pathway upon SOCS3 deficiency, which consequently activated the Wnt/ß-catenin pathway via targeting C/EBPß. Furthermore, applying a specific miR-155 antagonist or the autophagy agonist rapamycin efficiently suppressed tumor growth and eMDSC infiltration in vivo.
Conclusions Overall, these findings indicated that SOCS3 deficiency blocked autophagy-dependent myeloid differentiation of e-MDSCs via the miR-155/C/EBPß/Wnt axis, and thus targeted therapy against this pathway could be a potential therapeutic target in breast cancer.

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Immune cell types

533 CROSS-SPECIES IMMUNOGENOMIC ANALYSIS IDENTIFIES PATHWAYS OF CANINE NATURAL KILLER CELL RESPONSE TO CYTOKINE THERAPY, AND REVEALS CONVERGENCE OF ACTIVATED DOG AND HUMAN NATURAL KILLER TRANSCRIPTOMES

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Background Natural killer (NK) cells are key effectors of the innate immune system, but major differences between human and murine NK cells impede translation. Outbred dogs offer an important link for NK-based cancer immunotherapy studies. We compared gene expression profiles of dog NK signatures in vitro and from a phase I clinical trial of inhaled IL-15, and analyzed dog, mouse and human NK cells using a novel orthologous transcriptome.
Methods We performed differential gene expression (DGE) using resting healthy donor CD5dim NK populations and following ex vivo activation using recombinant human (rh) IL-15 or co-culture with irradiated feeder cells. Eight dogs with naturally-occurring pulmonary metastases were enrolled on a Phase I clinical trial of inhaled rhIL-15 using a 3+3 cohort design with escalating doses of inhaled rhIL-15. Blood was collected from study dogs before, during, and after therapy. We compared DGE among healthy and cancer-bearing dogs and then across mouse, dog and human NK cells in resting and activated states using ~7000 1:1 orthologous genes.
Results DGE revealed distinct transcriptional profiles between the ex vivo resting, IL-15 and co-cultured canine NK cells. Among treated patients, hierarchical clustering revealed that in vivo NK cell transcriptional signatures grouped by individual dog, and not amount of time exposed to treatment. PCA showed in vivo profiles of the clinical responders were distinctly separate from the non-responding patients (PC1 38%, PC2 12%). Patient in vivo NK cell transcription profiles most closely resembled those of ex vivo resting NK cells and not IL-15 treated or co-culture activated (PC1 43%, PC2 19%), likely reflecting key differences in activation. In cross-species analysis, PCA showed within-species spatial clustering of resting NK cells. After activation, variance between dog and human NK cells decreased, while variance between human and mouse NK cells increased (PC1 40%, PC2 28%).
Conclusions In this first transcriptomic sequencing of dog NK cells, we demonstrate distinct gene profiles of ex vivo activated NK cells from healthy donors compared to circulating NK cells from dogs receiving inhaled rhIL-15 on a clinical trial. Baseline in vivo NK cell profiles appear to predict response to therapy more than changes over time. We also show distinct gene profiles of NK cells across the most commonly used mouse, dog, and human NK populations, with convergence of dog and human NK cells after activation. By defining the canine NK cell DGE signatures, these data fill a gap in translational NK studies.
Ethics Approval The canine clinical trial study was approved by IACUC and Clinical Trials Review Board (Inhaled IL-15 Immunotherapy for Treatment of Lung Metastases, Protocol #20179).

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