between B16 melanoma and Mφs. These hybrids retained nuclear IRF3 translocation but failed to express IFN-β indicating a dominant-negative mechanism. Sequencing revealed no mutations in the IFN-β promoter. However, ATAC-sequencing indicated the IFN-β promoter remained epigenetically closed after STING pathway activation in B16 tumor cells, unlike Mφ controls. NF-κB is an innate immune pathway proposed to control IFN-β locus accessibility. We found B16 tumor cells and hybrids were defective in NF-κB nuclear translocation, unlike Mφ controls. Furthermore, tumor cells expressed normal levels of most NF-κB factors but over expressed the negative regulator IκBα. Combined treatment with cycloheximide and a STING agonist caused IκBα degradation and tumor cell-intrinsic IFN-β expression.

Conclusions

We find tumor cells fail to express IFN-β downstream of STING pathway activation. This is likely due to a dominant-negative inhibitor of NF-κB signaling, such as IκBα, which causes the IFN-β locus to remain epigenetically repressed in tumor cells and prevents IRF3 binding. We hypothesize restoring NF-κB signaling in tumor cells will enable tumor cell-intrinsic IFN-β expression and promote tumor rejection in vivo. Therapies based on identifying and reversing tumor-specific defects in IFN-β expression represent a strategy to induce tumor site-specific innate immune activation.

O61

Myeloid cell-targeted miR-146a mimic alleviates NF-κB-driven cytokine storm without interfering with CD19-specific CAR T cell activity against B cell lymphoma

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Background

NF-κB is a key regulator of inflammation, myeloproliferation and cancer progression, with an important role in leukemogenesis. Despite therapeutic potential, targeting NF-κB proved challenging. However, in non-malignant myeloid cells NF-κB activity is tightly regulated through many molecular mechanisms, including miRNA.

Methods

Here, we describe an original approach to NF-κB inhibition using miR146a, which targets upstream regulators of NF-κB signaling. We generated a myeloid cell-targeted NF-κB inhibitor by tethering a chemically-modified miR146a mimic oligonucleotide to a scavenger receptor (SR)/Toll-like receptor 9 (TLR9) ligand (C-miR146a).

Results

Unlike an unconjugated miR-146a molecule, C-miR146a was rapidly internalized and delivered to cytoplasm of target myeloid cells such as macrophages or myeloid leukemia cells. C-miR146a reduced protein levels of classic miR-146a targets, IRAK1 and TRAF6, thereby efficiently blocking NF-κB activation in target cells. Intravenous injections of C-miR146a mimic to miR-146-deficient mice prevented excessive NF-κB activation in myeloid cells, thereby alleviating myeloproliferation and exaggerated inflammatory responses to bacterial challenge. The NF-κB-driven release of IL-1 and IL-6 from monocytes is known to be responsible for cytokine release syndrome (CRS), which can occur in response to bacterial infections, antibody-based therapies and relatively frequently as a serious adverse effect of chimeric antigen receptor (CAR) T-cell therapies. While low expression of miR146a has not yet been implicated in CRS, C-miR146a treatments did reduce pro-inflammatory activity of human monocytes, at the level of IL-1 and IL-6 production, induced by the CD19-specific but not by the naive CAR T cells in vitro. Repeated systemic administration of C-miR146a oligonucleotide alleviated human monocyte-dependent CRS in xenotransplanted B-cell lymphoma model without impeding the on-target therapeutic effects of CAR T-cells against lymphoma cells.

Conclusions

Our results demonstrate potential of using myeloid cell-targeted miR146a mimics for treatment of inflammatory diseases and prevention of potential side effects of immunotherapies. The SR/TLR9-targeted miR-146a mimic design provides an outline for the development of miRNA therapeutics for a variety of myeloid cell-related diseases.

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Immuno-Conjugates and Chimeric Molecules

O62

Targeting myeloid tumors by Off-the-Shelf NK cells using an NKG2C-IL15-CD33 Trispecific Killer Engager

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Background

Allogeneic NK cell infusions can achieve remission in 30-50% of patients with relapsed/refractory acute myeloid leukemia (AML). This strategy may be limited by NK cell heterogeneity and lack of specificity. NK cells mediate antibody-dependent cellular cytotoxicity of tumors coated with therapeutic antibodies through the CD16 receptor. While a strong activation receptor, CD16 is also present on neutrophils, which represent a sink for CD16-driven therapies. To bypass this issue, we elected to target NKG2C, an activating receptor with better specificity which is present on more differentiated NK cells. CD94/NKG2C is a heterodimeric receptor that binds to HLA-E and associates with DAP12. NKG2C+ cells are enriched in AML patients who have undergone a hematopoietic stem cell transplant and reactivated CMV. [1] NKG2C+ NK cells are more responsive to AML and patients with higher circulating NKG2C+ cells have improved relapse free survival. [2]

Methods

We aimed to direct specific killing and proliferation of the NKG2C+ cells using a NKG2C1533 Tri-specific Killer Engager (TriKE), containing single chain variable fragments specific for NKG2C (on NK cells) and CD33 (on AML cells), and an IL15 moiety (to support NK cell survival and proliferation). An obstacle to utilizing NKG2C is the frequency of NKG2C+ cells, so we genetically modified induced pluripotent stem cells (iPSCs) to stably overexpress NKG2C alone or along with DAP12. We then differentiated these cells to NK (iNK) cells.

Results

In the presence of AML cell lines, NKG2C1533 TriKE activated NK cells from transplant patients with CMV reactivation, where NKG2C+ frequency correlated with activation (Figure 1A,B). The NKG2C1533 TriKE also led to preferential expansion of NKG2C+ cells (Figure 1C). We found that the NKG2C1533 TriKE mediated robust activation of NKG2C iNK cells and NKG2C/DAP12 iNK cells leading to THP1 cell killing (Figure 1D). The NKG2C/DAP12 iNKs exhibited the strongest response.

Conclusions

Engaging NK cells through NKG2C or genetically modified iNK cells expressing NKG2C, will be more specific than targeting through CD16, which will bind to CD16A on NK cells but also have off-target binding to CD16B on neutrophils. The NKG2C1533 TriKE is an effective way to selectively target NK cells with two applications, one in individuals with high frequencies of NKG2C+ cells and another within the iNK cell platform creating an "off-the-shelf" NK cellular therapy that is targeted, specific and efficacious where TriKE can be co-