Anti-PD-1 short-chain variable fragment triggers a potent NK cell response against AML tumor targets as compared to commercial full-length antibodies

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Abstract

Natural killer (NK) cells are large, granular lymphocytes that are critical component of the innate immune system. NK cells mediate a "first response" cytolitic effector function against virus infected cells and tumor transformed cells, as well as produce cytokines like interferon-γ which can enhance adaptive immune responses. These complex functions are regulated through a variety of activation and inhibition receptors that are well characterized, and have been extensively studied in the context of NK cell biology. NK cells uniquely attack cells that lack or have decreased expression of MHC class I antigens and express cell stress ligands. Due to their recognition and response to germ-line encoded ligands, NK cells kill tumor and virus infected cells without prior exposure or priming. However, various disease related immune subversion mechanisms have been observed that allow for evasion of the NK cell response and ultimately lead to adverse outcomes including tumor escape and disease progression.

Programmed death receptor-1 (PD-1) is an immune checkpoint regulator, predominantly expressed on T-cells, that potentiates the inhibition of T-cell activation and promotes immune regulation. In cancer, the production of PD-1 ligands (PD-L1/PD-L2) by tumor cells and their subsequent interaction with the PD1 receptor, leads to T-cell exhaustion and host immune suppression. Although subversion of the T-cell response through PD-1/PD-L1 interactions is well studied, little is known regarding PD-1 expression and regulation of NK cell function. While some studies have investigated the presence and role of PD-1 on NK cells, the data surrounding its expression and function is inconsistent. Evaluation of PD-1 expression using various clones of commercially available reagents generate different staining patterns further complicating consistent identification of the receptor. Thus, the level of expression, conditions of induction, and functionality in response to various tumor targets have not been extensively studied. Our lab has produced a novel antibody containing only the variable heavy and light chains of the clinical anti-PD-1 drug, pembrolizumab, called a short-chain variable fragment (scFv). This molecule demonstrates both strong staining and functional enhancement of NK cells in response to PD-1 ligand expressing targets. Our studies using this reagent identify a unique function of the scFv compared to the full length, commercially available antibodies. While the therapeutic benefit of targeting the PD-1 pathway is already proven and utilized in cancer immunotherapy, understanding the novel role of the PD-1 axis in NK cell biology can provide an additional focus for targeted therapy and deliver further immunotherapeutic strategies against cancer.

Conclusions

Our study on the anti-PD-1 scFv construct demonstrates superior target affinity and specificity as compared to commercial full-length anti-PD1 antibody. Using this scFv construct we show the expression of PD-1 receptor on all peripheral blood NK cells. Further, PD-1 blockade using the scFv construct shows enhancement of the cytolytic, cytokine production, and ADCC function of NK cells. Our data also reveals that the NK cell PD-1 pathway involves downstream signaling component (pAKT) parallel to that of the well characterized T-cell PD-1 signaling pathway. Finally, these results suggest a potential use for the anti-PD1 scFv in the development of novel BiKE/TriKE constructs that could be therapeutically used to enhance NK cell function in cancer immunotherapy.

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Figure 1: Structure and function of anti-PD1 scFv construct. (A) Anti-PD1 scFv was constructed using the variable heavy (VH) and light (VL) chains from the antigen-binding fragment (Fab) region of the full length anti-PD1 antibody, and connected by a short peptide linker. (B) The proposed anti-PD1 scFv construct binds to PD-1 receptors on NK and T-cells, preventing interaction with its biological ligands PD-L1/PD-L2. Blocking of PD-1 signaling prevents downstream inhibitory signals that eventually lead to T-cell exhaustion and NK cell dysfunction.