Tumor Antigen Expression-dependent Activation of the CD137 Costimulatory Pathway by Bispecific DART® Proteins

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Introduction

CD137 (4-1BB) is a costimulatory molecule expressed by activated T and NK cells that, upon interaction with its CD137 ligand, supports cell activation, proliferation and survival. Activation via CD137 holds great promise for cancer immunotherapy; however, current CD137 agonistic interventions are characterized with primary human T cells in the presence or absence of antigen-expressing cancer cells. To develop a therapeutic modality that reduces the potential for systemic CD137 effects, we applied the DART® bispecific platform to generate proteins that can induce tumor-antigen-dependent T-cell activation.

Methods: DART molecules were constructed containing anti-CD137 variable regions together with either anti-HER2 or anti-EphA2 variable regions. DART binding properties were evaluated by flow cytometry; signaling responses assessed using a NF-κB luciferase reporter cell line expressing CD137. Costimulatory activity was characterized with primary human T cells in the presence or absence of target tumor-antigen-expressing cells. Results: Flow cytometry analysis demonstrated that both HER2 x CD137 and EphA2 x CD137 DART molecules bind their respective target antigens. Co-culturing of a CD137- cells with tumor lines expressing HER2 or EphA2 revealed tumor-antigen-dependent CD137 pathway activation by HER2 x CD137 and EphA2 x CD137 DART molecules, respectively. To evaluate the effects of HER2 x CD137 and EphA2 x CD137 DART molecules on T-cell responses, costimulation T-cell assays were performed. In the presence of the relevant antigen-positive cell line, each respective DART molecule was able to promote T-cell proliferation and cytokine release in a HER2 or EphA2-dependent manner. No T-cell costimulation was observed by either DART molecule in the absence of antigen-expressing tumor cells. Furthermore, the level of tumor antigen-dependent costimulation supported by the DART molecules correlated with the level of tumor target expression. Consistent with the preferential induction of CD137 by the CD8 T-cell subset, CD137-based DART proteins induced a substantial increase in the fraction of CD8 centro-memory and effector memory T cells in the presence of the proper tumor-antigen-expressing cells. Conclusions: HER2 x CD137 and EphA2 x CD137 DART proteins promote T-cell costimulation in a tumor antigen-dependent manner and may provide an opportunity to target the CD137 costimulatory pathway for cancer immunotherapy, while limiting systemic T-cell activation and related side effects.

Results

Tumor-targeted CD137 DART Proteins Bind Each Antigen

Antigen-dependent Enhancement of T-cell Activation by HER2 x CD137 and EphA2 x CD137 DART Molecules

EphA2 x CD137 DART Induces EphA2-dependent Increase in the CD8 Memory T-cell Subsets

References


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