CONSTRUCTION AND USE OF DARPIN LIBRARY FOR THE DISCOVERY OF TUMOR SPECIFIC ANTIGEN BINDER PROTEINS.

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Adoptive transfer of tumor-specific T cells is proving to be an effective strategy for treating established tumors. In order to extend the specificity of T lymphocytes beyond their MHC-peptide complexes, a strategy has been developed that allows redirecting cytotoxic T cells to tumor cell surface antigens. One method of generating these cells is accomplished through engineering bulk T cell populations to express chimeric antigen receptors (CARs) which are specific for tumor antigens. This strategy combines an antigen-binding moiety, most commonly a single chain antibody, together with an activating immune receptor. However, single chain antibody typically shows low thermal stability and tends to denature or aggregate under the conditions of practical uses, thus limiting their biomedical and biotechnological applications. Tandem repeat proteins represent an alternative to single chain antibodies as a binding moiety within CAR. ~20 classes of such proteins are found in all forms of life where they participate in multiple protein-protein interactions. They are characterized by well defined topology and stability. Using a consensus designed ankyrin repeat protein (DARPin) with specificity for the tumor-associated antigen HER-2, we have generated anti-HER-2 DARPin CARs for expression on murine or human $CD8^+$ T lymphocytes. Our murine anti-HER-2 DARPin CAR is expressed on the surface of murine $CD8^+$ T cells, is functionally activated (production of IFN-γ and TNF-α) upon stimulation with HER-2, and induces specific killing of HER-2^+ tumor cells. We constructed a new designed ankyrin repeat protein (DARPin) library expressed in filamentous bacteriophage display system in a purpose to select binding proteins for known tumor-specific antigens and to discover new tumor-specific targets expressed on tumor cell surfaces. Currently the library is being screened with BCMA (B-cell maturation antigen) and Multiple Myeloma cell line.