Title: Protease-Activatable Nanorings for Selective Redirection of Immune Cells to Combat Solid Tumors

Authors: Kulkarni AS, Paul D, Ligori K, Rodriguez F, Rozumalski L, Friess R, Walcheck B, Wagner CR

The Wagner Lab has developed a protein nanoring based platform to redirect human immune (T and NK) cells to combat solid tumors. These nanorings are bispecific since they are comprised of tumor-targeting and immune cell-targeting dihydrofolate reductase (DHFR2) fusion proteins. These protein nanorings non-genetically functionalize the surface of immune cells and redirect them to target and destroy cancer cells. For treating solid tumors, we aimed to target the widely expressed antigen, epidermal growth factor receptor (EGFR). However, targeting EGFR could lead to potential “on-target, off-tumor” effects due to expression of EGFR on healthy tissue, particularly in skin. Therefore, to improve tumor specificity, we designed a histidine triad nucleotide binding protein-1 (HINT1) masked anti-EGFR nanobody-DHFR2 fusion protein. HINT1 was hypothesized to sterically block the binding to EGFR outside the tumor. The HINT1 steric mask and the anti-EGFR nanobody were fused through a tumor-associated protease sensitive linker. Therefore, protein nanorings made of HINT1-anti-EGFR-DHFR2 and anti-CD3-DHFR2 or anti-CD16-DHFR2 can non-genetically label the surface of T or NK cells, respectively. Once these nanoring-labeled immune cells enter the tumor microenvironment, cleavage of the HINT1 mask due to upregulated protease activity will result in the crosslinking of tumor and immune cells, leading to targeted tumor cell lysis. To this end, HINT1-anti-EGFR-DHFR2 protein was successfully designed, expressed, and purified. In vitro protease cleavage assay demonstrated the complete removal of the HINT1 mask within 24 hours. 2D cytotoxicity assays with T and NK-92-CD16 cells showed a potent lysis of EGFR+ A431 (human epidermoid carcinoma) cells. The EGFR-targeted lysis was diminished in the presence of HINT1 mask, thereby providing a proof-of-concept for the steric masking of the anti-EGFR nanobody. On-going efforts to deduce the stability and efficacy of these protease-activatable nanorings will be presented. We believe that our tumor-specific immune cell redirection platform can be clinically translated into an efficacious and safe immunotherapy for the treatment of EGFR+ solid tumors.