Background

Cancer-targeted therapies are often undermined by the heterogeneity and genetic instability of cancer cells. In addition, the cancer microenvironment (neoangiogenesis, pericytes and stromal cells) forms barriers protecting cancer cells. In contrast to tumor cells, the cells forming the tumor microenvironment have a low mutational rate and less phenotypic and genotypic heterogeneity. In particular, tumor endothelium is an attractive and accessible target for imaging and anticancer agents.

We and others identified TEM1 as a tumor target selectively expressed on tumor vascular and stromal cells in ovarian cancer and other cancer types, using genome-wide expression profiling and histological staining studies in ovarian cancer and other solid tumors. Moreover, TEM1 has been implicated in promoting angiogenesis and metastasis. Thus, TEM1 can be a tumor vascular marker with potential theranostic efficacy towards multiple cancer types.

We have previously isolated human TEM1-specific single chain variable fragment (scFv) antibodies using screening yeast-display recombinant scFv libraries. One of the scFv antibodies, scFv78, or ‘78F, binds to the extracellular domain of TEM1 (aa 234-390) of human and murine TEM1 with Kd of 2 nM.

Compared with IgG, scFv78 have shorter circulation time (renal clearance within hours or less) and are more sensitive to inactivation upon conjugation. Thus, we aim to optimize the pharmacokinetics and targeting features of anti-TEM1 scFv78 by genetic engineering to improve their avidity, circulation and tolerance to conjugation of payloads. These novel affinity ligands will be used in the project and beyond for targeting diagnostic and therapeutic payloads to ovarian cancer and other cancers.

Hypothesis

The project goal in the long term, is to develop theranostic reagents from scFv78-derived proteins with high affinity and specificity against tumor vascular marker TEM1. We have hypothesized that we can achieve this by genetic engineering of scFv78 with human IgG Fc fragment.

There are several advantages in producing recombinant eukaryotic fusion proteins for fusion proteins with the Fc region for rabbits, chickens and CHO of human IgG. (1) The Fc region has an affinity to proteins A and G, which enables affinity purification. (2) The scFc-Fc fusions are usually produced with an antibody-like dimerized structure, the biologically increases the affinity. (3) Fc-fused scFv are expected to have longer blood circulation and exert Fc-effector functions, both of which are advantageous for anti-tumor effects. Such fusion proteins are tested in vitro and in vivo as theranostic agents for ovarian cancer.

ImmunopET and Immunotoxogen Targeting Tumor Vasculature by Multivalent scFv Constructs against TEM1/CD248

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Conclusions

- High TEM1 mRNA level correlates with poor survival in two independent cohorts.
- Positive TEM1 staining was observed in 99.1% of ovarian cancer TMA containing 216 cores of tumor and stromal tissues, with no positive staining seen in control.
- Gene-activated scFv78 variants fused with Fc produced multimers. 78Fc and 78Fcab are in dynamic form, whereas 78Fc is in a dimerization mode and 78Fcab in tetrameric form.
- The affinity of scFv78-multimeric variants was higher compared to maternal 78 (Kd 21 nM from 2-10 M).
- scFv78-multimeric variants are serum and thermal stable.
- The 78Fc has much longer blood PK than maternal 78, thus is a much favorable candidate for imaging or therapy.
- 78Fc is tested in vitro and in vivo as TEM1-targeted imaging agent and as immunotoxin. We anticipate that anti-TEM1 78Fc fusion derivatives will be useful affinity ligands targeting ovarian and other cancer vasculature.

References

- George Cokus and Chunsheng Li. ANTI-TUMOR ENDOHELIAL, MARKER 1 (TEM1) ANTIBODIES AND USES THEREOF. Provisional patent US790. U.S. Patent Number 6160,505.