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Selection, optimization and characterization of anti-FGFR2 scFv and scFv-Fc antibody fragments and their conjugation with cytostatics

ABSTRACT

At present, the mainstay of treatment for most cases of cancer is systemic treatment that includes the use of cytotoxic agents. However, the therapeutic activity of most anticancer drugs in clinical use is limited by their general toxicity to proliferating cells, including some normal cells. A growing understanding of the molecular events that mediate tumor growth and metastases has led to the development of targeted therapies that offer the higher efficacy and limited toxicity to normal tissue. The proper choice of the molecular target, as well as the targeting molecule, are the two key factors in the development of an effective targeted therapy. Fibroblast growth factor receptor 2 (FGFR2) is one of the promising candidates for cancer therapy targets, as it is known to be overexpressed in multiple types of cancer, such as gastric, colorectal, breast, and endometrial cancer.

In this study, we used the phage display technology to produce a panel of high affinity single chain variable fragments (scFvs) against the extracellular ligand-binding domain of FGFR2 (ECD_FGFR2). The binders were selected from the human single chain variable fragment scFv phage display libraries Tomlinson I + J and displayed high specificity and binding affinity towards human FGFR2 with K_D values in nanomolar range. Based on the best clone selected (scFvF7), we performed the affinity maturation procedure and reformatted scFvF7 to bivalent formats of diabody and single-chain variable fragment fused with Fc-region (scFvF7-Fc) to improve recognition of FGFR2.

As a part of the work we engineered two types of conjugates – scFvF7 and scFvF7-Fc - with the drug monomethyl auristatin E (MMAE), synthetic strong cytostatic which is a derivative of the naturally occurring peptide in the marine shell-less mollusc *Dolabella auricularia*, inhibiting cell division by blocking tubulin polymerization. The scFv conjugates with the cytotoxic drug are analogous to the antibody-drug conjugates (ADC), intensively studied lately. ADCs offer multiple

advantages over classical monoclonal antibody targeting, namely greater efficacy of lower doses of drugs and more efficient and specific destruction of cancer cells.

The following part of the study focused on the confirmation of potential antitumor activity of the conjugates. We performed the analysis of their effect on the viability of tumor cells characterized by the overproduction of ligand, relative to control cells. The scFvF7-Fc-MMAE conjugate demonstrated very prominent cytotoxic effect, causing a decrease in FGFR2-positive cell viability up to 5%. The resulting IC_{50} values varied from 0.89 to 7.7 nM, depending on the cell line. The scFvF7-MMAE conjugate also showed relatively high cytotoxicity with IC_{50} values ranging from 7.7 to 49.4 nM against cell lines with the FGFR2 overexpression.

In conclusion, the scFv-based conjugates obtained in this work have the potential for use in anti-cancer therapies.