

Selection of antibody fragments against fibroblast growth factor receptor 1 for anticancer therapy

ABSTRACT

Purpose: A targeted delivery of active molecules with antibodies specific for tumor associated antigens represents the most important goal in present oncology research. Fibroblast growth factor receptor 1 (FGFR1) was shown to be a high-frequency targetable oncogene specific for smoking-associated lung cancers, identified in over 20% of cases of lung squamous cell carcinoma. Here, we describe the development of a fully human antibody fragment in scFvFc format and its potent cytotoxic conjugate designed to efficiently target FGFR1-positive lung cancer cells.

Experimental Design: Antibody phage technology was used to select a high affinity antibody fragment against the extracellular domain of FGFR1(IIIc). An enzyme immunoassay (ELISA) and surface plasmon resonance (SPR) analysis were applied for screening and characterization of individual antibody variants. The best binder (named scFvD2) was cloned to scFv *diabody* and Fc-fusion formats. The tumor-targeting properties of D2 antibody fragments were tested by detailed internalization studies in cancer cell lines using confocal microscopy. Antibody fragment scFvD2Fc was further modified by reduction of four interchain disulfides, followed by conjugation to monomethyl auristatin E (MMAE) using valine-citrulline proteolytic linker.

Results: All D2 antibody formats showed high affinity to FGFR1 with dissociation constants values of 18 nM (scFvD2), 0.82 nM (scFvD2 diabody) and 0.59 nM (scFvD2Fc). Obtained scFvD2 module was found to be exquisitely selective for FGFR1 versus other FGFR family members, and was able to bind FGFR1 even in the presence of natural ligand FGF2, as shown by competitive analysis. Confocal microscopy experiments revealed that scFvD2Fc was rapidly and specifically internalized by endocytic pathway into U2OS model cells overexpressing FGFR1 (U2OS-R1), whereas no scFvD2Fc uptake was observed by FGFR1-negative U2OS cells. Highly efficient uptake of scFvD2Fc was also present in lung cancer cells harboring oncogenic *FGFR1* gene amplifications: NCI-H1581 and DMS114. Based on scFvD2Fc we have developed scFvD2Fc-vcMMAE, a potent antibody-drug conjugate (ADC). Mass spectrometry analysis revealed that the conjugation process results in homogeneous ADC with a drug-to-antibody ratio of exactly 4:1. scFvD2Fc-vcMMAE was shown to undergo specific internalization by U2OS-R1 cells. *In vitro* studies demonstrated that scFvD2Fc-

vcMMAE effectively inhibited the growth of U2OS-R1 model cells, and NCI-H1581 and DMS114 lung cancer cells with EC50 values of 17 nM, 33 nM and 53 nM, respectively. Preliminary studies using immunocompetent mice have shown good tolerability of scFvD2Fc-vcMMAE *in vivo*.

Conclusion: In this study, we evaluated the scFvD2Fc as a vehicle for the targeted delivery of a potent toxin MMAE. Our results demonstrate that scFvD2Fc-vcMMAE possesses favorable characteristics and could provide an effective system for specific delivery of cytotoxic drugs into lung cancer cells overexpressing FGFR1.