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Antibody fragments specific for the fibroblast growth factor (FGF1) and its receptor, FGFR1

PhD thesis

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Abstract

Targeted therapy refers to an approach to block the growth and spread of cancer by interfering with specific molecules that are critical for tumor development and progression. By aiming only for cancerous cells, they offer the advantage of reducing the side effects characteristic for systemic cytotoxic therapy. Fibroblast growth factors (FGFs) and their receptors (FGFRs) regulate crucial biological processes such as cell proliferation and differentiation. Aberrant activation of FGFRs by their ligands and FGFR overexpression can promote tumor growth and angiogenesis in many tumor types, including lung and breast cancer. The development of FGF/FGFR axis-targeting molecules with potential implications for the therapy of FGF- and FGFR-driven tumors has been recently considered as a promising approach in the treatment of cancer.

In this dissertation phage display selection from Tomlinson I and J libraries was used to find scFv antibody fragments selectively binding FGF1 and preventing it from binding to its receptor. The selected scFv clones were expressed and characterized with regard to their binding to FGF1 with the use of ELISA, BLI and NMR methods. In the next step the scFvs were cloned to scFv-Fc format, as dimeric Fc fusions prove beneficial in prospective therapeutic application. As expected, bivalent scFvs-Fc exhibited significantly increased apparent affinity towards FGF1, as proved by kinetic parameters assessment with BLI measurements. *In vitro* experiments performed on the FGFR-expressing cell lines confirmed the ability of selected antibody fragments in scFv format to interfere with FGF1-induced activation of signaling cascades. Strong antiproliferative activity of the scFvs and scFvs-Fc in the *in vitro* cell models was also observed.

As a second approach to the FGF-FGFR interaction inhibition, peptides were fused to the Fc domain of an antibody to generate peptibodies that were specific to FGFR1. The peptide sequences were derived from FGF2 and the peptibodies architecture was designed and optimized. Subsequently, the proteins were expressed and thoroughly characterized in the context of binding the protein of interest. *In vitro* analysis on FGFR1-positive cell lines confirmed peptibody R to have the highest potential to inhibit the FGFR1-dependent signaling pathways. Moreover, in fluorescent microscopy experiments all generated peptibodies have proven to specifically bind as well as internalize and colocalize with FGFR1 in FGFR1-overexpressing cells.

Developed here specific antibody fragments and peptibodies serve as novel FGF-FGFR interaction inhibitors and can be utilized as powerful tools to use in the studies on further development of clinically relevant FGFR-targeted treatments.