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Selection and optimization of peptide antagonists of the FGF1-FGFR1 interaction

Summary

Fibroblast growth factor receptor 1 (FGFR1) is a protein involved in activating key signaling pathways. The activation of the receptor is done through its interaction with ligands and one of the ligands that binds with FGFR1 is fibroblast growth factor 1 (FGF1). Binding of this ligand to the receptor activates signaling pathways that affect cell proliferation, angiogenesis, and cell differentiation. Disturbances in the natural activity of FGFR1 are a key element in the arising and development of many types of cancer. The most common receptor aberrations include its excessive amplification on the cell surface, point mutations or chromosomal fusions with other proteins. Disorders related to the abnormal activity of the FGFR1 receptor occur the most frequently in lung and breast cancer.

Inhibition of FGFR1 activation is one of the methods used in therapies targeting FGFR1-dependent tumors. To date, the FDA has approved two drugs: erdafitinib (*Balversa*) and pemigatinib (*Pemazyre*) that target FGFR, and can be used in targeted therapy. Both drugs are selective, small molecule inhibitors that target the intracellular tyrosine kinase domain of FGFR. An alternative are potential drugs that target the extracellular domain of FGFR or its ligands, which are already being tested in the early stages of clinical trials. This group includes, for example, antibodies, which production is unfortunately associated with high costs. Moreover, most antibodies require special transport and storage conditions, which also increase costs and limit their range of applications. Therefore, the optimal solution seems to be searching for molecules that will be characterized not only by good kinetic parameters and a stable structure, but also by low production costs, ease of transport and trouble-free storage. One of the candidate drugs that meets most of these criteria are peptides. The advantages of these molecules are their fast and relatively cheap production (depending on the chain length and modification) along with easy transport and storage (e.g. in a lyophilized form). Well-optimized peptides are also characterized by significant selectivity and a strong affinity toward the molecular target.

The aim of doctoral project was to search for peptides, which disrupt the interaction between FGFR1 and FGF1. The *Phage Display* technique was used to select specific peptides. It relies on carrying out several selection rounds in the course of which, the pool of phage clones presenting peptides interacting with the antigen, is gradually narrowed. A commercially available Ph.D.™ C7C cyclic peptide phage display library (New England Biolabs) and the Ph.D.™ 12 linear peptide phage display library (New England Biolabs) were used to perform the selection.

At the first stage, the selections of phage display libraries of cyclic and linear peptides targeting extracellular domain of FGFR1 or FGF1 were performed. Then, the selected phage clones were checked in the ELISA assay in order to choose those with the strongest affinity to the antigen. Selected phage clones were sequenced to determine the order of amino acid residues in the target-binding peptides. The peptides of such an appropriate sequence were then

synthesized, according to the Fmoc strategy. Peptides selected basing on a phage display library of cyclic peptides were cyclized by the creation of disulfide bridge between the cysteine residues located in their structure. Cyclic and linear peptides were purified by reversed-phase high-performance liquid chromatography and their molar mass was confirmed by mass spectrometry. The obtained peptides were then lyophilized and dissolved in dimethylsulfoxide (DMSO). In this form, the peptides have been tested in cellular assays to activate FGFR1-dependent signaling pathways in the NIH3T3 (mouse embryonic fibroblasts) cell line that express all types of FGF receptors. This allowed the selection of the cyclic peptide F8 (R°F8), which had interacted with the extracellular domain of FGFR1 and thus weakened FGF1 – FGFR1 interaction. At the next stage, the R°F8 peptide in the cyclic and linear form was subjected to proliferation tests carried out on the BAF/3 FGFR1c cell line (murine interleukin-3 dependent pro-B cell line) - which does overexpress the FGFR1 isoform IIIc on its surface, and on the BAF/3 cell line, which did not expose any of the FGF receptors. The results of cell proliferation tests performed on the BAF/3 FGFR1c cell line confirmed that the cyclic peptide RºF8 decreased cell viability alongside with increasing concentration (1-80 μM). Furthermore, the experiments on the BAF/3 cell line have showed that the cyclic peptide RºF8 interacts specifically with FGFR1c. It should be emphasized that the results of peptide cytotoxicity tests performed on the BAF/3 FGFR1c cell line have proved that the cyclic peptide RºF8 is not toxic to cells in the tested concentration range meaning that the observed decrease in their viability results from the ability of RºF8 to inhibit the FGF1-FGFR1 interaction. The results of cell proliferation assays performed on the BAF/3 FGFR1c cell line have also confirmed, that the cyclic form of the RºF8 peptide is necessary to possess the desired biological activity.

Summarizing the results presenting in this dissertation, it should be stated that the screening of phage display libraries of cyclic and linear peptides targeting FGFR1 and FGF1 has allowed the selection of the cyclic peptide R°F8, which weakens FGF1 – FGFR1 interaction. It is worth noting that the selected RºF8 peptide is the first cyclic peptide described in the literature that acts as an antagonist of the FGF1-FGFR1 interaction. It was also confirmed that the cyclic conformation of this peptide is necessary to maintain the desired biological activity.