Daria Nawrocka

Abstract

Biological activity of human fibroblast growth factor 2 (FGF2) covalent dimers

Fibroblast growth factor 2 (FGF2) is one of the best-characterized members of the FGF protein family. It plays an important role in cell proliferation, migration, and differentiation, as well as processes such as angiogenesis and wound healing. The signal transmitted by FGF2 to cells occurs through complex interactions with specific transmembrane receptors of the tyrosine kinase family (FGFR1-4), as well as heparin or proteoglycans. Its effector functions are triggered by the formation of a dimeric complex, 2:2:2 FGF2-FGFR1-heparin.

To facilitate receptor activation and improve the biological properties of FGF2, I developed covalent dimeric ligands of FGF2. Wild-type FGF2 mutations were designed to create variants with a single reactive cysteine exposed on the protein surface for chemical conjugation through a maleimide-thiol reaction with bis-functionalized linear PEG linkers. I designed eight variants of FGF2 dimers with specific topologies, differing in the relative orientation of individual FGF2 molecules. The engineered proteins remained functional in terms of FGFR activation and subsequent ERK1/2 signaling pathway activation. Additionally, they exhibited increased stability, mitogenic potential, anti-apoptotic activity, and more effectively induced migratory responses in normal fibroblasts, compared to FGF2 monomers. Importantly, the biological activity of the dimers was significantly less dependent on exogenous heparin administration. Furthermore, some dimeric FGF2 variants demonstrated more efficient internalization into FGFR-overexpressing cancer cells. The results were published in the *International Journal of Molecular Sciences* in 2020.

Based on these findings, I identified one variant of the FGF2 dimer as the most promising for use as a drug carrier in FGFR1-targeted therapy, due to its exceptional ability for efficient and selective internalization into cells with FGFR1 overexpression. The FGF/FGFR pathway represents an optimal molecular target in anti-cancer therapies due to its

fundamental role in the regulation of key processes. Aberrations in the FGF/FGFR signaling axis are associated with various pathologies, including amplifications or mutations in FGFR genes, or changes in transcriptional and translational regulation leading to receptor overproduction on the surface of cancer cells. Molecular alterations in FGF receptors in cancer cells affect their growth, proliferation, angiogenesis, and invasiveness.

In the next stage of my doctoral work, I developed a highly cytotoxic conjugate consisting of the FGF2 dimer and two potent cytotoxic drugs with completely independent mechanisms of action - α-amanitin and monomethyl auristatin E. The drugs were specifically attached to the dimer using enzymatic ligation methods involving SnoopLigase and sortase A enzymes. The resulting dimeric conjugate with two strong cytotoxic drugs selectively bound and internalized into FGFR1-overexpressing cancer cells. Cytotoxicity studies showed that the developed dual conjugate of the FGF2 dimer with α-amanitin and monomethyl auristatin E exhibited approximately 10-fold higher toxicity against FGFR1-overexpressing cell lines compared to equimolar mixtures of individual monomeric conjugates with α-amanitin or monomethyl auristatin E.

The application of two cytostatic drugs with different mechanisms of action, penetration through biological membranes, and susceptibility to active efflux from cells can be useful in the treatment of heterogeneous tumors and demonstrate greater effectiveness against cells with a high potential for acquiring drug resistance. The results of this part of the study were published in the *International Journal of Molecular Sciences* in 2023.