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Intracellular binding partners of fibroblast growth factor 1 (FGF1)

ABSTRACT

FGF1 is the best described member of the fibroblast growth factor family. It acts mitogenically, inducing DNA synthesis in many cell types, and takes part in many physiological processes like stimulation of cell migration, angiogenesis and wound healing. FGF1 transmits signal into cells through specific FGF receptors (FGFR1-4), with tyrosine kinase activity. Transphosphorylation of cytoplasmic domain of the FGF receptors leads to the activation of three main signalling cascades: PLCγ/PKC, PI3K/Akt and Ras/MAPK, that are involved in a wide range of cellular responses, including: cell survival, motility, proliferation, differentiation and apoptosis.

In addition to the classical extracellular activity of FGF1, its unique feature among other growth factors is the ability to cross the cell membrane and to translocate into the cytosol and nucleus. It has been shown previously that FGF1 is transported through the cell membrane by endocytosis after being bound to one of two specific FGF receptors (FGFR1 or FGFR4). The translocation from endosomes into cytosol requires activity of several proteins such as: PI3K, p38 kinase and HSP90 as well as the presence of membrane potential generated by proton pumps.

The mechanism of translocation is partially described, but the role of FGF1 inside the cell remains unknown. One of the methods to address the growth factor's intracellular function is the identification of its intracellular binding partners, what was the main aim of this thesis.

Following methods of identification were combined: yeast two-hybrid screen (Y2H) and two types of analysis of protein complexes using mass spectrometry (MS). Altogether, twenty novel intracellular proteins interacting with FGF1 were identified. For selected proteins, their direct interaction with FGF1 was confirmed by pull-down assays and SPR measurements. Interestingly, 10 out of 20 proteins found are involved in processes related to cell viability, such as apoptosis, cell proliferation, and cell cycle regulation. The function of FGF1 binding partners allowed to propose the role of intracellular FGF1 for the first time.

The hypothesis on anti-apoptotic function of FGF inside the cell was verified for FGF1, as well as for FGF2, which is the member of the same subfamily. It has been shown that exogenous FGF1 and FGF2 added to fibroblast cells in the presence of the specific inhibitors of FGFR kinase activity (PD173074 lub SU5402) inhibit apoptosis induced by

serum starvation, staurosporine or p53 activators (tenovin-6 and NSC348884). Application of the range of translocation inhibitors (geldanamycin, radicicol, SB203580 and bafylomycin A1) confirmed that anti-apoptotic activity of FGF1 and FGF2 is a consequence of their translocation into the cell.

Moreover, special attention was paid to one of the identified binding partners of FGF1, nucleolin, which may play a role in the process of translocation as it is involved in the nucleo-cytoplasmic trafficking. It was shown that nucleolin is essential for FGF1 phosphorylation by PKCδ in the cell nucleus, what is the key signal for FGF1 export into cytosol.

Altogether, in this work 20 novel FGF1 binding partners were identified. While analyzing the interaction of one of them, nucleolin, with FGF1, the mechanism of FGF1 nuclear export was partially explained. In addition, it was shown that translocation of both FGF1 and FGF2 provides completely independent signal from the receptor activation, which protects the cells from apoptosis and promotes their survival in stress conditions.