Title

Role of fibroblast growth factors 1 and 2 (FGF1 and FGF2) in apoptosis process

Abstract

Fibroblast growth factors 1 and 2 (FGF1 and FGF2) belong to a protein family consisting of 22 vertebrate proteins. FGF1 and FGF2 bind to specific cell-surface receptors (FGFRs), activating intracellular signalling pathways: phospholipase Cγ and protein kinase C (PLCγ/PKC pathway), phosphoinosite 3-kinase and Akt kinase (PI3K/Akt pathway), as well as protein Ras and mitogen associated kinases (Ras/MAPK pathway). Receptor activation by fibroblast growth factors stimulates proliferation, migration or differentiation of many cell types. FGF1 and FGF2 are endocytosed in the complex with FGFR and are translocated through the endosomal membrane into cytosol and nucleus of the cell. This is a unique process, which was observed for just several other proteins. Despite many years of research, the role of translocated FGF1 and FGF2 remains unclear. Translocation is not a constitutive process but it is induced by stress factors, including growth factors deprivation or oxidation stress. Interestingly, endogenous FGF1 and FGF2 can display an antiapoptotic function. Moreover, several intracellular proteins involved in apoptosis process were identified by proteomic approaches as potential binding partners of FGF1 and FGF2. This suggests the involvement of translocated FGF1 and FGF2 in cell survival.

The aim of this study was verification of a hypothesis that FGF1 and FGF2 proteins translocated into cytosol and nucleus are related to apoptosis process.

Iinteraction of FGF1 and FGF2 with p53, MDM2, PCAF, UACA, Sirt1 and CDK4 was confimed using pulldown and surface plasmon resonance techniques. The results indicate that FGF1 and FGF2 may form complex with these proteins inside the cell and suggest the involvement of the growth factors in cell survival.

Antiapoptotic activity of exogenous FGF1 and FGF2 was verified. Apotosis was analyzed using two quantitive methods (annexin V and 7AAD staining, as well as measurement of caspase 3/7 activity and cell viability) and imaging *via* microscope.

To separate the biological effect of FGF1 and FGF2 translocation into cells from the effect of receptor activation, experiments were perfomed using FGFR kinase activity inhibitors. Inhibition of serum starvation-induced apoptosis was observed when exogenous FGF1 and FGF2 were added to NIH 3T3 cells. It was also observed that staurosporine-induced apoptosis was inhibited by exogenous FGF1 and FGF2 in BJ or NIH 3T3 cells. Moreover, antipoptotic activities of FGF1 and FGF2 were also exhibited against p53-dependent apoptosis in NIH 3T3 cells.

To verify the impact of translocated FGF1 and FGF2, translocation of the growth factors through the endosomal membrane was blocked using: geldanamycin, radicicol, SB203580 and bafilomycin A1. In the presence of each of the inhibitors mentioned and simultaneous inhibition of FGFR activity no antiapoptotic activity of exogenous FGF1 and FGF2 was observed. Furthermore, experiments with monensin were performed to reverse translocation-blocking activity of bafilomycin A1 and revealed apoptosis inhibition by exogenous FGF1 and FGF2. The results indicate that the antiapoptotic activity of exogenous FGF1 and FGF2 is connected with the presence of the growth factors in cytosol and nucleus after translocation process.

In addition, it was shown that enogenous FGF1 and FGF2 produced by transiently transfected HEK 293 and U2OS cells also inhibit staurosporine-induced apoptosis. The results confirm that FGF1 and FGF2 present inside the cell exhibit antiapoptotic activities.

This study shows that translocation of FGF1 and FGF2 into cytosol and nucleus inhibits apoptosis induced by various factors. Taking into account that FGF1 and FGF2 translocation process is stimulated by stress conditions, the promotion of cell survival is possible to be the role of translocated FGF1 and FGF2 into cytosol and nucleus of the cell.