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The Insulin-Like Growth Factor-I Receptor and Apoptosis

Implications for the Aging Progress

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Introduction

Apoptosis or programmed cell death is a process that is a genetically controlled response for cells to commit suicide, and is orchestrated by mechanisms involving various proteins. The physiological role of apoptosis is to kill unwanted cells. During development of the nervous system for example, a large excess of redundant neurons die off during the period when synapses are being formed between the neurons and their target cells *(1)*. A second important role for apoptosis is as a defense mechanism whereby prevention of the spread of viruses to neighboring cells is achieved *(2)*. It is also critical for the normal functioning of the immune system, where certain cells need to be removed, otherwise they may attack normal cells. In preventing the onset or progression of tumors, apoptosis is the antithesis of cellular proliferation and can impair cellular proliferation *(3)*. Finally, apoptosis is a distinctive feature of the aging process, in which it leads to loss of essential cells and the impairment of normal functioning of specialized organs.

Apoptosis is a highly ordered process that may be characterized by nuclear changes including chromatin condensation, fragmentation, and margination as well as internucleosomal DNA cleavage, which gives rise to the characteristic DNA laddering *(4)*. In addition, there is cytoskeletal disruption, shrinkage of the cell and membrane blebbing with membrane-bound apoptotic bodies. The process often results in an undesirable inflammatory response.

Insulin-like growth factors (IGF-I and IGF-II), like several other growth factors, prevent apoptosis in a number of different cells and tissues *(5–7)*. The mechanisms involved in these effects are not well defined. In this review the authors describe the current knowledge of the signaling pathways whereby IGF-I inhibits apoptosis.

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The Map Kinase and PI 3'-Kinase Pathways

The IGFs exert their biological effects by binding and activating the IGF-I receptor, which is ubiquitiously expressed. The initial response to the binding of the ligand is autophosphorylation of the receptor followed by tyrosine phosphorylation of two major substrates of the receptor, namely insulin receptor substrate (IRS)-1 and IRS-2. Tyrosyl-phosphorylated IRS-1 and IRS-2 then interact with a number of src homology 2 (SH2) domain-containing proteins (Fig. 1). These include p85, the regulatory subunit of phosphoinositide (PI) 3'-kinase; the tyrosine phosphatase PTP1D (or Syp); the gaunine-nucleotide exhange protein Grb2; the Ras-binding protein GAP; and the protooncogene products Crk, and Nck *(8)*. Shc is also phosphophorylated directly by the IGF-I receptor and binds the Grb2/Sos complex. These interactions lead to activation of downstream signaling proteins and kinases; all arranged as signaling cascades. For the IGF-I-mediated response, the significant cascades are the Ras/Raf/mitogen-activated protein (MAP) kinase pathway and the PI 3'-kinase pathway *(9)*.

To determine the possible role of these signaling pathways in the action of IGF-I on the inhibition of apoptosis, the authors utilized the well-characterized model of differentiated pheochromocytoma PC12 cells. These cells differentiate in the presence of serum and nerve growth factor (NGF), and then undergo apoptosis following the removal of serum and NGF. Apoptosis is clearly seen as DNA laddering, or using the TUNEL assay. Apoptosis, as measured by DNA laddering, is inhibited by the addition of physiological concentrations of IGF-I (10nM).

The Ras/Raf/MAP kinase pathway may be inhibited by multiple interventions. Expression of dominant negative proteins will inhibit activation of downstream enzymes, thereby disrupting the normal signaling cascades. For example, transient transfection of an expression vector encoding a dominant negative MAPK/Erk kinase (MEK)

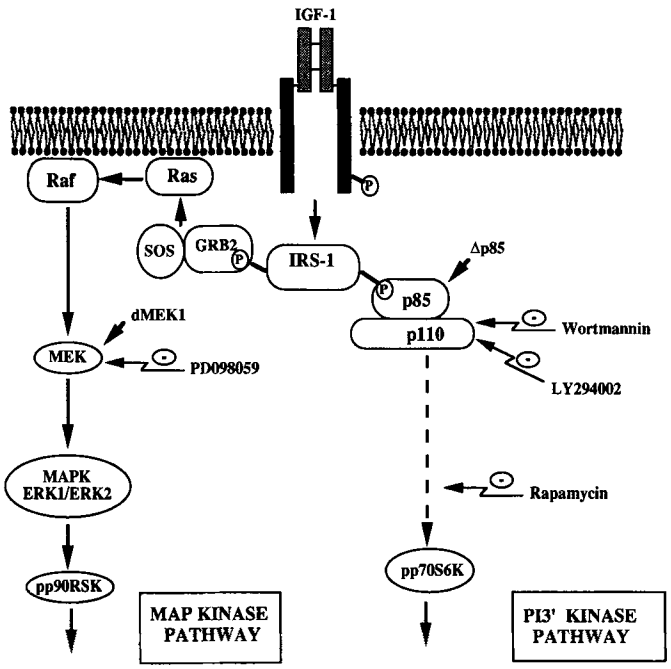

Fig. 1. IGF-I receptor signaling pathways involved in apoptosis. Wortmannin and LY 294002 inhibit the PI3'-Kinase pathway whereas PD098059, a specific MEK inhibitor, affects the MAPK pathway. Δp85 and dMEK 1 are dominant negative inhibitors of PI3'K and MAPK, respectively.

protein into differentiated PC12 cells abrogated the effect of IGF-I on inhibiting apoptosis. A complementary technique by which to disrupt the crucial signaling cascades is the use of synthetic inhibitors of several of these kinases. PD098059 specifically inhibits MEK and similarly inhibits IGF-I's biological effect on inhibition of apoptosis *(10–12)*. Cell survival has also been determined by manual cell counting and the estimation of cell number using an MTT assay. Survival of PC12 cells, as determined by both of these methods, closely paralleled the responses to apoptosis as measured by DNA laddering and in situ staining for apoptotic nuclei.

The role of the PI 3'-kinase pathway was also investigated using a dominant negative p85 molecule stably expressed in PC12 cells *(13)*. The blockade of PI 3'-kinase diminished the IGF-I protective effect from apoptosis. The fungal metabolite wortmannin and the synthetic compound LY294002, one a general inhibitor and the other a specific inhibitor of the p110 catalytic subunit of PI 3'-kinase, inhibited IGF-I protection from apoptosis *(14)*.

Thus, in differentiated PC12 cells both the Ras/Raf/MAP kinase and the PI 3'-kinase pathways are apparently involved in mediating the anti-apoptotic IGF-I action. Since these pathways also interact with other signaling cascades, the results of the studies cited do not exclude the role of other similar signaling pathways such as the p38 and JNK stress-related pathways. Another critical protein in the apoptotic pathway is the recently identified proto-oncogene product Akt (also called protein kinase B-α or RAC-α), which contains a pleckstrin homology (PH) domain. Based on the known property of the PH domains of other proteins to bind lipids, Akt is a potential downstream target for PI 3'-kinase. Activation of Akt occurs in response to increased lipid kinase activity of PI 3'-kinase following stimulation by growth factors. Furthermore, the PI 3'-kinase inhibitors wortmannin and LY294002 block growth factor-activation of Akt. Specifically, IGF-I enhances Akt activity 20–50 fold with concommitant phosphorylation of residues Thr 308 and Ser 473 of Akt. Primary cultures of cerebellar neurons undergo apoptosis in the absence of survival factors. Addition of physiologic concentrations of IGF-I inhibits this apoptosis. The Ras/Raf/MAP kinase pathway is not involved in mediating this protective effect. However, IGF-I stimulates activation of PI 3'-kinase, Akt and p70S6 kinase, and each of these activities is inhibited by wortmannin. However, p70S6 kinase is apparently not important in mediating the effect of IGF-I since rapamycin fails to inhibit the IGF-I effect on preventing apoptosis *(15,16)*.

Thus in certain cell lines both the PI 3'-kinase and MAP kinase pathways are involved in IGF-I's anti-apoptotic effects, whereas in other cells it appears only the PI 3'-kinase/Akt or p38 pathways mediate the protective effect.

The BCL-2 Family of Survival/Death Proteins

To determine whether the IGFs may inhibit apoptosis via this family of proteins, we studied human 293 embryonic kidney cells that undergo apoptosis in serum-free medium. IGF-I induced the expression of bcl-xl in a time- and dose-dependent manner. An increase of bcl-xl mRNA preceded an increase in protein levels *(12)*. In SH-SY5Y neuroblastoma cells mannitol-induced hyperosmotic stess is associated with alterations in the levels of the bcl-2 and bcl-xl proteins *(17)*. The reduction in the levels of these anti-apoptotic proteins can be overcome by treatment with IGF-II or overexpression of the IGF-I receptor. These results support the hypothesis that long-term effects of the IGFs on preventing apoptosis may be mediated via these proteins.

Thus, in both 293 and SH-SY5Y cells the proteins of the Bcl-2 family are involved in the inhibition of apoptosis mediated by activation of the IGF-I receptor and its down-stream signaling pathways. Again, the authors suggest that in each cell type specific pathways are involved in modulating apoptosis and, futhermore, these pathways may also be specific for the particular growth factor mediating the effect.

Other proteins that may transduce the effects of the IGFs, include the ICE-related proteases, of which there are several described members. Yama/CPP32 and ICE/LAP3 are cleaved and activated upon hyperosmotic stress in SH-SY5Y cells *(17)*. This cleavage is prevented by IGF-II

treatment. Activation of these serine proteases is dependent on cleavage of the precursor ICE proteases. Cleaved ICE proteases subsequently cleave the 116 kDa nuclear protein poly (ADP-ribose) polymerase (PARP) yielding a 85-kDa apoptotic protein fragment. PARP protein fragments are thus yet another marker of apoptosis. The exact IGF-I receptor-activated pathways that integrate at the level of the ICE proteases have yet to be fully described; however, studies to date indicate that the mechanisms of the antiapoptotic effect of the IGFs is multifaceted and it is likely that the signaling via the ICE proteases is also complex in its regulation.

Conclusions

This review has described results of recently published reports on the signaling pathways involved in IGF-I inhibition of apoptosis. As this is an exciting and topical subject with importance to the understanding of a number of physiological and pathological states, one can anticipate a large number of reports in the near future, predictably with many more pathways being described. It seems obvious that a complete understanding of the mechanisms involved in the IGFs anti-apoptotic effect will elucidate the complex interactions occuring within the cell and potentially guide the design of therapeutic agents with high benefit-to-risk ratios. However, the eminently more important biological question to be understood is the balance between cell cycling resulting in cellular proliferation and apoptosis. The perturbation of this programmed balance is critical in the process of aging.

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