Regulation of apoptosis by insulin and IGF-1

Author: Pierre De Meyts
Author title: MD, PHD, F.A.C.E.

Novo Nordisk Pharmatech A/S

An increase in the number of cells growing in cell culture is the result of two opposing effects: an increase in the number of cells that traverse the cell cycle and divide into two daughter cells (mitosis), or a decrease in the number of cells that die according to different modalities, the most prominent one being apoptosis (1), or both.

Insulin and IGF-I are both capable of facilitating cell progression through the cell cycle, and of inhibiting apoptotic mechanisms.

In this section we will review the mechanisms that control apoptosis, while regulation of the cell cycle is discussed in another section.

Although there were earlier descriptions of some of the cell morphological changes associated with programmed cell death, the term apoptosis was first used in the seminal paper published in the British Journal of Cancer in 1972 (2), by John Foxton Ross Kerr (born 1934), Professor of Pathology at the University of Queensland (Australia), Alastair A. Currie (1921-1994), Professor of Pathology at the University of Aberdeen (Scotland), where Kerr was on sabbatical, and his graduate student Andrew H. Wyllie (later Professor of Pathology at the University of Cambridge). In 2002, the Nobel Prize in Physiology and Medicine was given to Sydney Brenner (born 1927), a South African biologist at the Laboratory of Molecular Biology in Cambridge (UK), John E. Sulston (born 1942), a British biologist at University of Cambridge (UK), and to H. Robert Horwitz (born 1947), biologist at MIT in Boston (USA), for their work on the genetic regulation of organ development and programmed cell death in the worm *Caenorhabditis Elegans*.

This mechanism of controlled cell deletion is common to all multicellular organisms. Cells undergo marked morphological changes: blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation and chromosomal DNA fragmentation. Cells eventually split into small closed entities (apoptotic bodies) that can be phagocytosed by other cells, preventing the release of toxic cellular substances that may cause necrosis. Apoptosis is triggered by a variety of environmental stimuli, both physiological and pathological including stress. The intracellular mechanisms involved in apoptosis are very complex (Fig. 1); while most proteins involved in the apoptotic network are known, much work remains to be done to unravel their interactions.

Figure 1: Mechanisms of apoptosis. Below you can see a schematic view of the three main apoptotic pathways: the intrinsic pathway, the extrinsic pathway and the granzyme pathway. See text for explanation. Adapted from ref. 2.

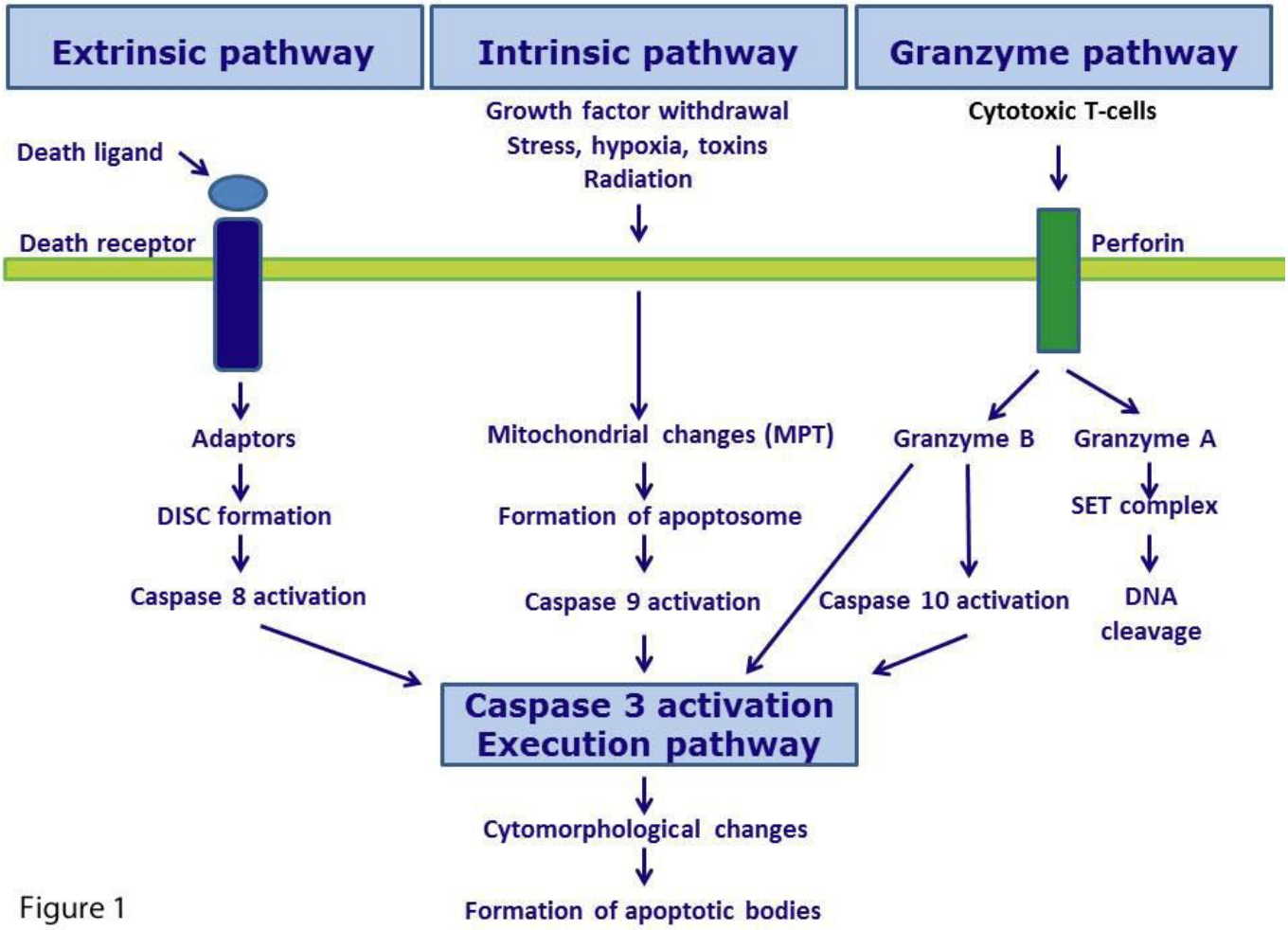


Figure 1

We will here only briefly summarize the salient features of the apoptotic process, see ref. 3 for more detailed review. Some cells apoptose through extrinsic pathways that involve death receptors such as Fas (fatty acid synthetase) or TNF (tumour necrosis factor) receptors; others have a default death pathway that must be blocked by a survival factor such as a hormone or a growth factor (3). Serum withdrawal is a classic way to initiate this pathway. In the end, apoptosis is an energy-dependent process that involves the activation of a group of cystine proteases called "caspases" and involves a complex cascade of events that link the initiating stimuli to the death of the cell (3). The two main regulatory

mechanisms used by extracellular signals are either by targeting mitochondrial functionality (intrinsic pathway) or by directly transducing the signal via adaptor proteins to the apoptotic mechanisms (extrinsic pathway). There is an additional pathway mediating T-cell-induced cytotoxixity and perforin-granzyme (a serine protease) A or B-dependent killing of the cell (Fig. 1).

The mitochondrial pathway involves an increased mitochondrial permeability resulting in release in the cytosol of cytochrome c and SMACS (small mitochondria-derived activators of caspases) that bind to and desactivate IAPs (inhibitors of apoptosis proteins). IAPs repress the caspases. Mitochondrial permeability is regulated positively or negatively by 25 members of the Bcl-2 family of proteins (4), under the control of the tumor suppressor proteinp53.

Cytochrome c and ATP released from the mitochondrial intermembrane space form the apoptosome consisting of ATP, apoptosis protease-activating factor (APAF)-1, cytochrome c and caspase-9, which becomes activated by autoproteolytic cleavage and activates the execution caspase-3.-6 and -7, which leads to th collapse of cellular infrastructure (7).

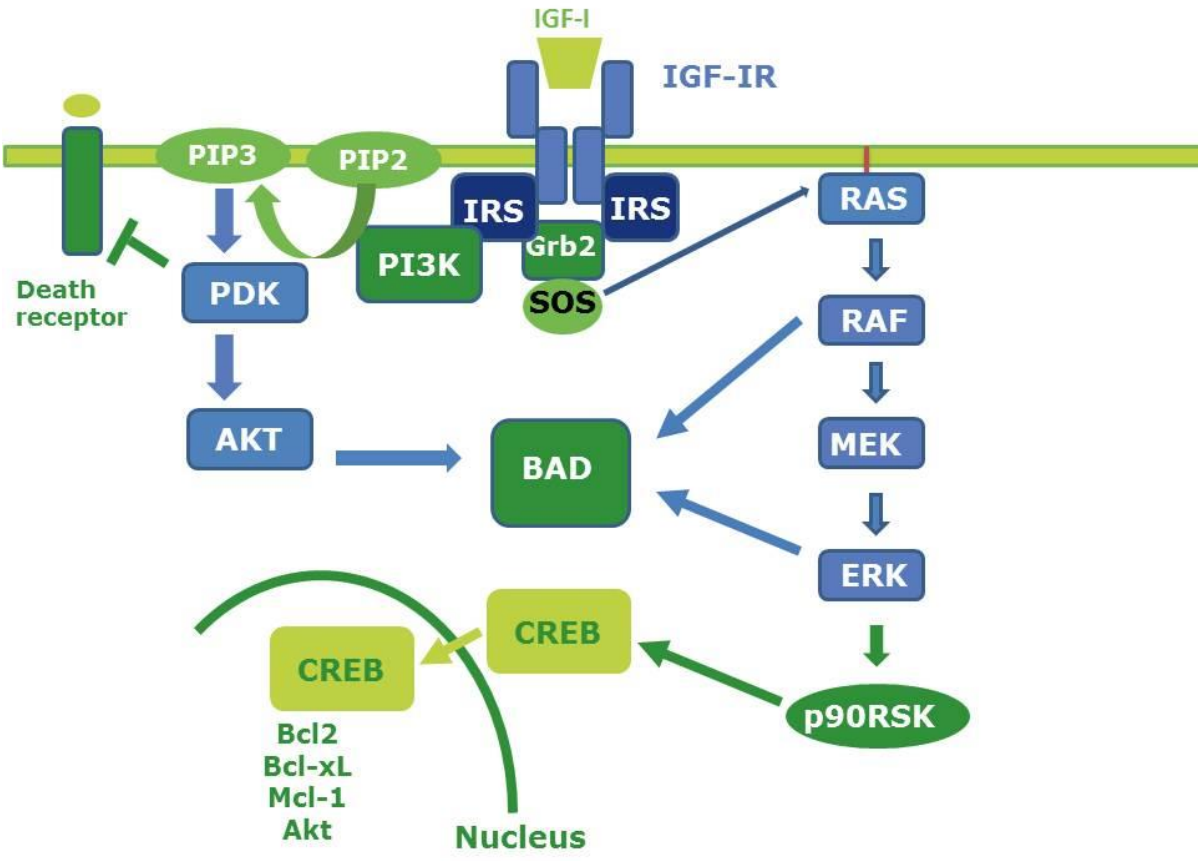
The extrinsic pathway involves binding of trimeric ligands to their receptors which cluster (FasL to the FasR or TNFa to the TNR1). Binding of FasL to FasR recruits the adapter protein FADD (Fas-associated death domain), while binding of TNFa to the TNR1 recruits the adapter protein TRADD (TNF receptor-associated death domain). TRADD then recruits FADD and RIP (receptor-interacting protein). FADD forms a death-inducing signalling complex (DISC) with procaspase-8 resulting in its autocatalytic activation (3) and triggering of the "execution phase".

The extrinsic, intrinsic and granzyme B pathways converge on the same terminal "execution" pathway, that is initiated by the cleavage of caspase-3 by caspases 8, 9 or 10. The granzyme A pathway activates a parallel, caspase-independent pathway via single stranded DNA damage (5).

There are many more players in the apoptosis cascades, see tables 1-4 in ref. 3.

IGF-I is a potent anti-apoptotic growth factor at low concentrations in multiple cell types (6,7). This anti-apoptotic effect involves both the PI-3K and MAPK/ERK1/2 pathways (Fig. 2) as shown by the use of specific inhibitors in serum-deprived PC12 cells (6), but other pathways also appear to be involved (for review, see ref. 7).

IGF-I anti-apoptotic signalling. Below you can see schematic views of the pathways whereby IGF-I inhibits apoptosis. See text and refs. 5 and 6 for explanation.
Abbreviations:
BAD: Bcl-2-associated death promoter.
CREB: cAMP response element-binding protein.
Bcl: B cell leukemia protein-
Mcl: Myeloid cell leukemia protein.



Many of the antiapoptotic actions of IGF-I appear to be through the regulation of mitochondrial membrane permeability via Bcl-2 proteins like Bad (7) via Akt. It also

appears capable of inhibiting the extrinsic apoptotic pathway via regulation of death-inducing receptors (7). These properties may hold therapeutic potential for hypoxic/ischemic brain injury, amyotrophic lateral sclerosis, Huntington disease, Alzheimer disease and cancer (7).

Numerous papers indicate that insulin exerts also a potent anti-apoptotic role in a variety of cell types (8-17). The experimental conditions (e.g. using high concentrations of insulin and IGF-I, and no dose-response curve) do not always allow to conclude whether the insulin effect is through the insulin receptor or the IGF-I receptor. In a few carefully controlled studies using native cells or insulin receptor-transfected cells (e.g. 11,12,16), the authors established unequivocally that insulin at physiological concentrations has anti-apoptotic effects through the insulin receptor.

The PI3K pathway appears to play a prominent role in this effect.

In a recent intriguing paper (8), the group of C. Ronald Kahn at the Joslin Diabetes Center in Boston showed that brown adipocytes from mice with both the insulin receptor and IGF-I receptor knocked out were resistant to apoptosis induced by serum deprivation. Sensitivity to apoptosis was restored by knocking in either receptor, which was then prevented by the appropriate ligand. These data suggested that the unoccupied insulin and IGF-I receptors are proapoptotic (in a kinase independent fashion) while the ligand-occupied receptors are antiapoptotic.

Finally we should mention that autophagy or autophagocytosis (a process involving the lysosomal degradation of a cell's own components), if dysregulated, can also lead to cell death (18). A recent study suggests that this process is also inhibited by IGF-I (19).

In summary, both IGF-I and insulin have antiapoptotic effects on multiple cell lines. Both the IGF-I and insulin receptors are capable of mediating this effect. Insulin at high concentrations in cell culture will thus protect cells against apoptosis through both the IGF-I receptor and the insulin receptor. Multiple signalling pathways are implicated but the PI3K pathway appears to play a prominent role.

REFERENCES

1. Galluzzi L, Maiuri MC, Vitale I, Zischka H, Castedo M, Zitvodel L et al. Cell death modalities: classification and pathophysiological implications. Cell Death Differ 14:1237-1243 (2007)

2. Kerr JFR, Wyllie AH and Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 26:239-257 (1972)

3. Elmore S. Apoptosis: a review of programmed cell death. Toxicol Pathol 35:495-516 (2007)

4. Cory S and Adam JM. The Bcl2 family: regulators of the cellular life-or-death switch. Nat rev Cancer 2:647-656 (2002)

5. Martinvalet D, Zhu P and Lieberman J. Granzyme A induces caspase-independent mitochondrial damage, a required first step for apoptosis. Immunity 22:355-370 (2005)

6. Párrizas M, Saltiel AR and LeRoith D. Insulin-like growth factor-I inhibits apoptosis using the phosphatidylinositol 3`-kinase and mitogen-activated protein kinase pathways. J Biol Chem 272:154-161 (1997)

7. Kooijman R. Regulation of apoptosis by insulin-like growth factor (IGF)-I. Cyt Growth fact Rev 17:305-323 (2006)

8. Boucher J, Marcotella Y, Bezy O, Mori MA, Kriauciunas K and Kahn CR. A kinase-independent role for unoccupied insulin and IGF-I receptors in the control of apoptosis. Science Signaling3 (151), ra87 (doi:10.1126/scisignal.2001173).

9. Tseng YH, Ueki K, Kriauciunas KM and Kahn CR. Differential roles of insulin receptor substrates in the anti-apoptotic function of insulin-like growth factor-1 and insulin. J Biol Chem 277:31601-31611 (2002)

10. Diaz B, Pimentel B, De Pablo F and De La Rosa EJ. Apoptotic cell death of proliferating neuroepithelial cells in the embryonic retina is prevented by insulin. Eur J Neuroscience 11:1624-1632 (1999)

11. Bertrand E, Atti A, Cadoret A, L'Allemain G, Robin H, Lascols et al. A role for nuclear factor Kappa B in the antiapoptotic function of insulin. J Biol Chem 273:2931-2938 (1998)

12. Lee-Kwon W, Park D, Baskar PV, Kole S and Bernier M. Antiapoptotic signalling by the insulin receptor in Chinese hamster ovary cells. Biochemistry 37:15747-15757 (1998)

13. Rampalli AM and Zelinka PS. Insulin regulates expression of C-fos and c-jun and suppresses apoptosis of lens epithelial cells:Cell Growth Diff 6:945-953 (1995)

14. Kang S, Song J, Kang H, Kim S, Lee Y and Park D. Insulin can block apoptosis by decreasing oxidative stress via phosphatidylinositol 3-kinase and extracellular signal-regulated protein kinase-dependent signalling pathways in HepG2 cells. Eur J Endocrinol 148.147-155 (2003)

15. Tanaka M, Sawada M, Yoshida S, Hanaoka F and Manurouchi T. Insulin prevents apoptosis of external granular layer neurons in rat cerebellar slice cultures. Neurosc Lett 199:37-40 (1995)

16. Johnson D, Bernal-Mizzachi E, Alejandro EU, Han Z, Kalynyak TB, Li H, Beith JL, Gross J, Warnock GL, Townsend RR, Permutt MA and Polonski KS. Proc Natl Acad Sci USA 103:19575-19580 (2006)

17. Iida KT, Suzuki H, Sone H, Shimano H, Toyoshima H, yatoh S, Asano t, Okuda Y and Yamada N. Insulin inhibits apoptosis of macrophage cell line, THP-1 cells, via phsphatidylinositol 3-kinase-dependent pathway. Artherioscler Thromb Vasc Biol 22:380-386 (2002)

18. Corcelle EA, Puustinen P and Jäättelä M. Apoptosis and autophagy: targeting autophagy signalling in cancer cells - "trick or treats"?FEBS J276:6084-6096 (2009)

19. Troncoso R, Vicenzio JM, Parra V, Nemchenko A, Kawashima Y, Del Campo A, Toro B, Battiprolu PK, Aranquiz P, Chiong M, Yakar S, Gillette TG, Hill JA, Abel ED, LeRoith D and Levandero S. Energy-preserving effects of IGF-I antagonize starvation-induced cardiac autophagy. Cardiovasc Res 93:320-329 (2012)