Cell Survival and Apoptosis

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Regulation of survival signals from the insulin-like growth factor-I receptor

R. O'Connor, C. Fennelly and D. Krause
Cell Biology Laboratory, Department of Biochemistry, University College Cork, Lee Maltings, Cork, Ireland

Abstract

Suppression of apoptosis by survival factors is important for the maintenance of normal tissue homoeostasis and the response to infection or injury. Survival factors such as insulin-like growth factor-I (IGF-I) initiate a signalling cascade that starts by tyrosine phosphorylation of substrates leading to the activation of serine kinases that modulate the activity of members of the Bcl-2 family, which regulates the apoptotic machinery in most cells. Tumour cells often have enhanced survival mechanisms due either to up-regulation of the IGF-I receptor and its ligands or to loss of function of a phosphatase (PTEN) that regulates part of this survival pathway. The C-terminus of the IGF-I receptor appears to be a regulatory domain for the anti-apoptotic activity of this receptor, and certain residues within the C-terminus are essential for this regulatory activity. Knowledge of the proteins and pathways, which interact with these C-terminal domains, should lead us to ways of modulating IGF-I-mediated survival in tumours.

Introduction

Our understanding of the role of peptide hormones and cytokines as necessary survival factors for most cells has come from our understanding of the process of cell death (apoptosis). Genetic and biochemical evidence supports the idea that the apoptosis programme is a default pathway during the critical points of a cell's life, such as development, cell division, in response to viral infection or oncogene activation, and in response to toxic stimuli. All of the effectors and regulator proteins necessary for apoptosis are present in cells at all times, and the process is only kept in check by anti-apoptotic stimuli from sources such as survival factors and anti-death genes.

The necessity of maintaining cell survival by suppression of apoptosis has been conserved during evolution from the nematode *Caenorhabditis elegans* [1]. The proteins that regulate apoptosis in *C. elegans* are analogous to the Bcl-2 family [2], the caspase family members [3] and Apaf-1 [4]. In this system, 131 cells out of a total of 1090 somatic cells are programmed to die in order for a normal worm to develop. The functioning of the *ced-9* gene (*bcl-2* homologue) is essential for *C. elegans* to develop, and it is required to suppress apoptosis in cells that are needed to survive for development [5]. The activity of *ced-9* is blocked by the *egl-1* gene product [6], which is homologous to the mammalian BH3 (Bcl-2 homology 3)-domain-containing members of the Bcl-2 family, which contains Bak and others [7,8]. Ced-9 in turn blocks the activity of ced-4 or its mammalian homologue Apaf-1, which is essential for activation of ced-3, a mammalian caspase homologue [9].

The apoptotic programme outlined above is kept in check by survival signals that come either from cell surface contacts or from soluble survival factors such as insulin-like growth factor-I (IGF-I). A precise balance between survival signals, death signals and the regulation of the core cell death machinery is essential for the development and maintenance of healthy cells (Figure 1). Regulating an intrinsic death programme by external survival signals also provides an innate defence against viral infection and against the survival of damaged cells that could give rise to

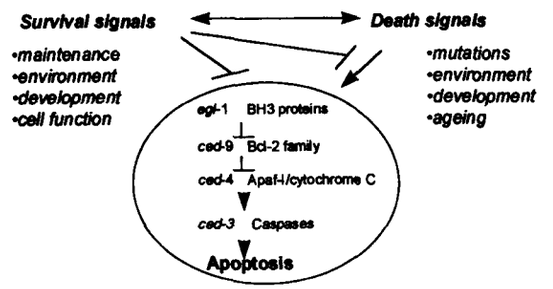
Key words: apoptosis, IGF-I, IGF-I receptor, phosphatases, survival factors.

Abbreviations used: IGF, insulin-like growth factor; IGF-IR, IGF-I receptor; PDK-1, phosphoinositide-dependent kinase 1; PI3-K, phosphoinositide 3-kinase.

Figure 1

Survival signals that suppress apoptosis

See the text for details.



tumours. However, as discussed below, viral infections and tumours do arise and this can be attributed in many cases to the up-regulation of anti-apoptotic (or survival) mechanisms in response to viral signalling or in response to oncogene activation.

IGF-I as a survival factor

IGF-I and IGF-II are single-chain peptides of approx. 70 and 67 amino acids respectively, which were initially characterized as having mitogenic actions as well as insulin-like activities in adipose and muscle tissue, and have overall identity with insulin of approx. 40–50% (reviewed in [10,11]). The IGFs are ubiquitously expressed and are produced in large quantities by the liver. Both IGF-I and IGF-II are found circulating in serum at high concentrations (100 nM) in association with high-affinity IGF binding proteins, which sequester them away from insulin receptors, facilitate their transport and regulate their biological functions at a cellular level. IGF-I has been recognized for a considerable period of time as an important mitogen for many cell types, but only more recently has its role in the suppression of apoptosis been appreciated. IGF-I protects fibroblasts that have been induced to die by activation of the c-*myc* oncogenes in media containing low serum [12] or overexpression of the pro-apoptotic Bcl-2 family member Bak [7]. It protects tumour cells and fibroblasts from chemotherapeutic drugs [13], caspase activation [14] or CD95 ligation [15]. Haemopoietic cells that are induced to die by interleukin-2 or interleukin-3 withdrawal are protected by IGF-I [16], and several kinds of neurons can be protected from stress by adding IGF-I [17].

The IGF-I receptor (IGF-IR) and apoptosis

All of the actions of IGF-I as a survival factor are mediated through the IGF-IR, which is a transmembrane tyrosine kinase receptor that becomes activated by binding of any of three ligands: IGF-I, IGF-II, or insulin at supra-physiological concentrations. The IGF-IR has been shown to mediate mitogenic, transformation and anti-apoptotic activities (reviewed in [10,11]), and has been shown to be absolutely essential for normal cell growth and development. Mice that have a targeted disruption of the receptor gene that leads to a null phenotype are 40% of normal size and die early in embryogenesis [18]. The IGF-IR contributes to survival in cells of different origin, including cells derived from the nervous system and from the mesenchyme [13,17]. Ectopic expression of the IGF-IR enhances the degree of IGF-I-mediated protection in FL5.12 cells and 32D cells [19].

Suppression of apoptosis by the IGF-IR is a distinct function from the receptor's mediation of mitogenic signals, since it occurs in the presence of cycloheximide, it does not require *de novo* protein synthesis [11], and it is separable from mitogenesis by mutational analysis of the receptor [19,20].

There is considerable evidence from mouse models and *in vitro* systems to support a role for IGF-IR-mediated anti-apoptotic activity in carcinogenesis, where it could suppress apoptosis that is induced by dominant oncogenes such as *myc* [11]. In a transgenic carcinogenesis model involving simian virus 40 T antigen targeted to the β-islet cells, activation of the IGF-IR by IGF-II is a critical determinant of carcinogenic progression [21]. Elevated levels of IGF-IR have been detected in human tumours of lung, breast and colon [22–24], and this is often associated with increased levels of circulating IGF-I and/or IGF-II. Antisense inhibition of IGF-IR expression suppresses tumorigenicity of tumour cells introduced into mice, and IGF-IR-blocking antibodies also inhibit tumour growth. All of these studies indicate that the IGF-IR could be a specific target at which to inhibit the survival of tumour cells and make them more susceptible to eradication or drug treatment.

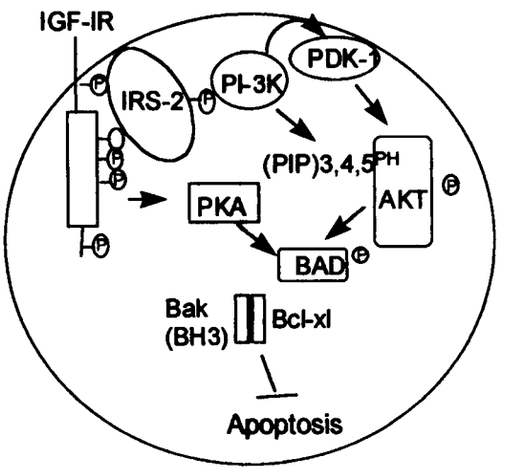
Survival signals from the IGF-IR

The survival signalling pathways from the IGF-IR are at least partially known (reviewed in [11]). Upon activation the IGF-IR becomes autophos-

Figure 2

Survival signalling pathway from the IGF-IR

Abbreviations: IRS-2, insulin receptor substrate-2; (PIP)3,4,5, PtdIns(3,4,5)P_3; PH, pleckstrin homology; PKA, cAMP-dependent protein kinase; BH3, Bcl-2 homology 3 domain. See the text for details.



phorylated on several tyrosines. It then recruits a large docking protein, insulin receptor substrate-2, which in turn can recruit the inositol lipid kinase phosphoinositide 3-kinase (PI3-K). Both of these substrates become phosphorylated by the IGF-IR. PI3-K activates a 3-phosphoinositide-dependent protein kinase called PDK-1 [25]. The lipid product of PI3-K, PtdIns(3,4,5)P_3, binds to the pleckstrin homology domain of the serine kinase AKT and this, together with PDK-1 phosphorylation, activates the AKT kinase [26–28]. AKT can subsequently phosphorylate a regulatory member of the Bcl-2 family, Bad [29], resulting in the sequestration of Bad away from the death suppressor Bcl-xL [30]. Bcl-xL is then thought to be free to remain in a complex with the caspase activator Apaf-1, thus preventing caspase activation and apoptosis [31] (Figure 2).

Regulation of IGF-IR survival signals

Now that at least one signalling pathway leading from the IGF-IR directly to the core cell death machinery is known, it is of great interest to understand what regulates this pathway. Kinase-regulated signalling pathways are regulated by phosphatases, two of which are known to function in the IGF-IR survival pathway (Figure 3). One of these is PTEN, which dephosphorylates PtdIns-(3,4,5)P_3 and thus reverses PI3-K-mediated activation of AKT [32]. The other phosphatase is calcineurin, which has been shown to dephosphorylate Bad and thus reverse the affects of AKT phosphorylation [33]. Interestingly, PTEN is the product of a tumour suppressor gene, because its gene locus on chromosome 10 (LOH 10q23) exhibits allelic loss and mutation in advanced cancers [34]. PTEN knockout mice are resistant to apoptosis and have increased AKT activity [32]. The PTEN phenotype illustrates the importance of the IGF-IR survival signalling pathway in the generation and progression of tumour cells.

Phosphatases that directly regulate the IGF-IR or its phosphorylation of immediate substrates are not known. A phosphatase that specifically regulates the IGF-IR would be a valuable target for manipulating the survival of many cell types, in particular neurons and tumour cells.

Figure 3

Regulation of survival signals

Phosphatases and putative phosphatases (?) are indicated in bold type. Abbreviations: IRS-2, insulin receptor substrate-2; (PIP)3,4, PtdIns(3,4)P_2; (PIP)3,4,5, PtdIns(3,4,5)P_3; PH, pleckstrin homology; PKA, cAMP-dependent protein kinase.

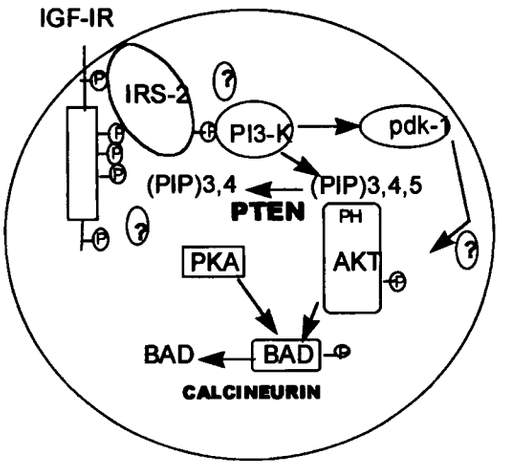
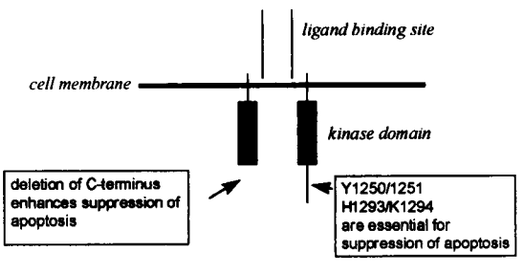


Figure 4

The C-terminus of the IGF-IR is a regulatory domain

See the text for details.



We have shown that the C-terminus of the IGF-IR is likely to be a regulatory domain for its anti-apoptotic activity (Figure 4). Deletion of the entire C-terminus or the last 40 amino acids in the receptor was sufficient to generate a receptor that was constitutively active for suppression of apoptosis [19]. This suggests that regulatory proteins are recruited to this extreme C-terminal region of the IGF-IR, one of which we have very recently identified as the cytoplasmic kinase CSK (D. Buckley, N. Spellacy, C. Fennelly, G. Loughran, D. Krause, A. Sant and R. O'Connor, unpublished work). Within the C-terminus of the IGF-IR there are two subdomains (at tyrosines-1250 and -1251 and at the histidine and lysine residues at positions 1293/1294) that are required for the anti-apoptotic activity of the IGF-IR (Figure 4).

To try and identify a role for these subdomains, expression constructs encoding the entire C-terminus and the C-terminus with mutations at these subdomains were generated. Expression of the C-terminal protein in MCF-7 tumour cells or fibroblasts resulted in induction of apoptosis, and this was reversed in the mutants [36,37]. Therefore the tyrosines at positions 1250/1251 and histidine-1293/lysine-1294 are required for the pro-apoptotic activity of the C-terminus when it is expressed as an isolated domain. Analysis of fragments that contained either one of these subdomains by transfection studies and *in vitro* kinase assays confirmed the importance of tyrosine-1250 and -1251. These studies also suggest that the C-terminus of the IGF-IR interacts with or regulates proteins that can suppress anti-apoptotic signalling from the receptor. It is not yet known what these regions of the receptor interact with or how they might regulate the receptor, but current efforts are focused on the signalling pathways that may be affected by the C-terminal proteins.

In conclusion, anti-apoptotic signals from the IGF-IR are likely to be critical for the maintenance of many types of cells, and are particularly important in the survival of tumours. Identification of the regulators that interact with the C-terminus of the receptor to control its anti-apoptotic activity could provide a way in which to manipulate the survival of cells.

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Role of the BH3 (Bcl-2 homology 3) domain in the regulation of apoptosis and Bcl-2-related proteins

R. J. Lutz
Apoptosis Technology, Inc., 148 Sidney St., Cambridge, MA 02139, U.S.A.

Abstract

The Bcl-2 family of proteins play a prominent role in the regulation of apoptosis. From the initial identification of *bcl-2* as an oncogene in follicular lymphoma through genetic studies in *Caenorhabditis elegans* to recent functional studies focusing on the importance of mitochondrial events in cell death signalling, the members of this protein family continue to be implicated in pivotal decision points regarding the survival of the cell. The family can be divided into two classes: those such as Bcl-2 and Bcl-xL that suppress cell death, and others, such as Bak and Bax, that appear to promote apoptosis. The Bcl-2 family is characterized by specific regions of homology termed Bcl-2 homology (BH1, BH2, BH3, BH4) domains, which are critical to the function of these proteins, including their impact on cell survival and their ability to interact with other family members and regulatory proteins. The identification of the BH3 domain as a potent mediator of cell death has led to the emergence of an additional family of pro-apoptotic proteins (such as Bad, Bik, Bid and Hrk) that share identity with Bcl-2 only within this death domain. These BH3-only proteins may be part of a regulatory network serving to integrate cell survival and death signals, an assertion that is supported by the identification of a BH3-only protein, Egl-1, as part of the central core of cell death signalling in *C. elegans*. While the mechanism of action of the BH3-only proteins remains unclear, recent studies on the regulation of critical protein–protein interactions and activity of Bad by phosphorylation in response to growth factor signalling suggest that the active state of BH3-only proteins may be regulated by post-translational modification. Additional modes of regulation, such as transcriptional, translational and subcellular localization, are also likely to be important.

Key words: Bad, BH3-only proteins, Bid, cell death signalling, pro-apoptotic proteins.
Abbreviations used: Ant, Antennapedia homeoprotein internalization domain; BH3, Bcl-2 homology 3; IL-3, interleukin-3; NGF, nerve growth factor.

Introduction

The ultimate fate of a cell is the outcome of an interplay between survival signals and signals that activate the cell death pathway. How are these signals integrated? What are the mechanisms that tip the balance from survival to death? The Bcl-2 family of cell death regulatory proteins appears to occupy a pivotal position in the control of this critical process [1]. The Bcl-2 family itself is composed of proteins with opposing functions. Certain family members, such as Bcl-2 and Bcl-xL, can suppress cell death induced by diverse death stimuli, whereas other members of the family, such as Bax and Bak, act to promote cell death. An additional property that is shared by these two groups is their propensity to heterodimerize with other family members. The Bcl-2 family is defined by conserved regions of identity called Bcl-2 homology (BH) domains. Family members share one to four of these BH domains, which in most cases have been shown to be critical to the regulation and activation of their function.

The importance of the BH3 domain as a potent mediator of cell death and protein binding function was first identified in studies aimed at determining the molecular requirements for the interaction of the pro-apoptotic Bcl-2 family member Bak with the death suppressor Bcl-xL [2]. The BH3 domain of Bak was uniquely required for its cell killing activity and its interaction with Bcl-xL. Furthermore, expression of small truncated derivatives of Bak containing the BH3 domain was sufficient for these activities [2]. Sequence analysis of other pro-apoptotic proteins