Growth Factors as Survival Factors: Regulation of Apoptosis

Mary K. L. Collins, Gordon R. Perkins, Gemma Rodriguez-Tarduchy, María Angela Nieto and Abelardo López-Rivas

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Summary

Apoptosis is now widely recognized as a common form of cell death and represents a mechanism of cell clearance in many physiological situations where deletion of cells is required. Peptide growth factors, initially characterised as stimulators of cell proliferation, have now been shown to inhibit death in many cell types. Deprivation of growth factors leads to the induction of apoptosis, i.e. condensation of chromatin and degradation in oligonucleosome-sized fragments, formation of plasma and nuclear membrane blebs and cell fragmentation into apoptotic bodies which can be taken up by neighbouring cells. Here we discuss the mechanism(s) by which growth factors may inhibit apoptosis.

Apoptosis: an overview

Often referred to as programmed cell death, apoptosis has been extensively reported in the scientific literature through morphological studies in many tissues where death was taking place as a consequence of a physiological phenomenon (reviewed in ref. 1). In these studies the presence of scattered single cells with highly condensed chromatin has been one of the parameters indicating the presence of apoptotic cell death⁽²⁾. In a later phase the chromatin appears to be distributed into sub-cellular structures called apoptotic bodies⁽³⁾.

However, apoptosis is not only observed in the balanced situation of physiological tissue turnover but also in the elimination of specific cell subsets that occurs in embryogenesis, for instance in the embryonic development of the intestine and nervous system and in the regression of female sexual organs in the male⁽⁴⁾. Apoptosis has also been implicated as the mechanism of cell elimination during tissue regression in metamorphosis⁽⁵⁾. Apoptotic cells have also been observed during tumour growth and regression. Thus, for example, apoptotic bodies of epithelial cell origin were described during development of squamous cell carcinoma⁽⁶⁾ and kinetics studies have demonstrated waves of apoptosis during sarcoma growth⁽⁷⁾. Therefore, during tumour growth some cells, perhaps those deprived of growth factors, undergo apoptosis. When tumours are treated with chemotherapy, apoptosis is responsible for at least some of the cell deaths which ensue⁽⁸⁾.

There are further experimental modulations of tissues where cell death is induced and apoptosis is observed. Regression of hyperplastic organs such as the liver or kidneys after lead poisoning is accompanied by the appearance of apoptotic cells⁽⁹⁾. Apoptosis is observed in the rat prostate after repeated withdrawal of testosterone stimulation⁽¹⁰⁾, in rat liver after removal of tumour promoters⁽¹¹⁾, in keratinocytes after UV irradiation⁽¹²⁾ and in neuronal cells following glutamate treatment⁽¹³⁾.

Because apoptotic cells are rapidly, and specifically, engulfed *in vivo* by neighbouring cells⁽¹⁴⁾ in order to keep cell debris to a minimum and to allow shrinkage of a tissue without disruption of its basic architecture, these morphological tissue studies probably greatly underestimate the number of deaths occurring. Indeed, it has been proposed that many cells in the normal animal are undergoing apoptosis, and that those which fail to enter this cell death program are rescued by essential 'survival factors'⁽¹⁵⁾. This has been most conclusively demonstrated for glial cells of the rat optic nerve. If the peptide factor platelet-derived growth factor (PDGF) is supplied to the developing nerve, a decrease in the number of apoptotic oligodendrocyte progenitor cells and an increase in the final number of oligodendrocytes are observed⁽¹⁶⁾. More mature oligodendrocytes are dependent on the supply of ciliary neurotrophic factor (CNTF) from the neurons which they myelinate and apoptose in its absence⁽¹⁷⁾. Developing neuronal cells themselves die in the absence of nerve growth factor (NGF), produced by the target cells which they innervate⁽¹⁸⁾, and administration of exogenous NGF to prenatal rats decreases the death of sympathetic neurons⁽¹⁹⁾. Thus, one can imagine that many cells are kept alive by a complex network of paracrine survival factors, the levels of which control the balance of cell populations. The purpose of this review is to discuss the mechanism by which factor deprivation can lead to apoptosis and to describe situations in which peptide growth factors act as survival factors.

Growth factor deprivation

The mechanism by which growth factor removal can lead to apoptosis can best be studied in cells in culture. A wide variety of cells maintained in culture have been shown to undergo apoptosis when deprived of essential growth factors. These include vascular endothelial cells deprived of fibroblast growth factor (FGF)⁽²⁰⁾, mouse embryo cells after epidermal growth factor (EGF) removal⁽²¹⁾, rat pheochromocytoma PC12 cell line and sympathetic neurons deprived of NGF⁽²²⁾, hormone-dependent cells of the breast or prostate deprived of steroids^(23,24) and glial cells deprived of PDGF⁽¹⁶⁾. In the hemopoietic and immune systems there are numerous reports of the induction of a cell death program by apoptosis after withdrawal of specific growth factors. It was first described⁽²⁵⁾ that interleukin-2 (IL2)-dependent T lymphocytes enter a program of endonuclease activation and cell death by apoptosis when IL-2 is removed from the culture medium. Germinal centre B lymphocytes also enter apoptosis in the absence of antigen receptor triggering⁽²⁶⁾. A similar mechanism seems to operate to regulate the development of all cells of the hemopoietic system from multipotential pre-

Fig. 1. Signalling pathways leading to apoptosis during growth factor deprivation.

cursors in bone marrow. This has been demonstrated in several interleukin-3 (IL3)-dependent progenitor cell lines^(27,28), in activated erythroid progenitor cells after erythropoietin removal⁽²⁹⁾ and in primary cultures of bone marrow after their removal from the marrow microenvironment⁽²⁸⁾.

The mechanism by which growth factor removal may lead to apoptosis is represented in Fig. 1. The signal transduction pathways and patterns of gene expression maintained in the presence of diverse growth factors are clearly not identical. They will however all decline upon removal of factor, which will lead in many cases to a decrease in basic cell functions, such as metabolic rate⁽³⁰⁾. A change in the pattern of gene expression in the cell will also occur. Recent experiments have demonstrated that an 'imbalance' in expression of genes involved in the stimulation of proliferation by growth factors may be responsible for entry into apoptosis upon factor removal. These studies demonstrate that constitutive expression of c-myc in fibroblasts results in cells which apoptose rather than arrest upon serum removal⁽³¹⁾. High levels of myc are also expressed by activated hemopoietic cells, and additional myc expression leads to more rapid apoptosis in IL3-dependent cells⁽³²⁾.

Whether new gene expression is required for apoptosis following factor removal remains unclear. For example, protein synthesis inhibitors slow the rate of death in some hemopoietic cell lines deprived of IL3⁽²⁷⁾, but protein or RNA synthesis inhibitors do not affect entry into apoptosis in the murine IL3-dependent cell line BAF3, when IL3 is removed⁽²⁸⁾.

Transformed cells may be those which either proliferate upon growth factor removal, or fail to undergo apoptosis in the absence of factor. The oncogene *bcl-2* was first identified as being translocated to the immunoglobulin locus, and therefore over-expressed, in a class of B cell lymphomas⁽³³⁾. Transgenic mice over-expressing the *bcl-2* gene product in T or B lymphocytes show greatly reduced apoptosis of these cells^(34,35). Transfection and over-expression of the *bcl-2* gene in hemopoietic cell lines inhibits apoptosis following IL3 removal⁽³⁶⁾, its introduction into neurons prevents apoptosis upon withdrawal of NGF⁽³⁷⁾, and it inhibits apoptosis when serum is removed from fibroblasts constitutively expressing c-myc⁽³⁸⁾. The *bcl-2* gene product is a 30 kDa protein localised partly in mitochondria⁽³⁶⁾ and also associated with other cell membranes⁽⁵⁰⁾. How the protein inhibits cell death is unknown, but it inhibits a variety of apoptotic pathways (see also below), suggesting that it may act at a late, common step. The down-regulation of expression of 'survival genes', including *bcl-2*⁽³⁶⁾, may be an event that follows growth factor removal; however we have demonstrated that loss of *bcl-2* expression does not precede apoptosis when IL3 is removed from BAF3 cells⁽⁴⁰⁾. The tumour suppressor gene *p53* is probably involved in the stimulation of apoptosis when at least some growth factors are removed, as loss of wild-type *p53* function allows factor-dependent cells to escape apoptosis on IL6 removal⁽⁴¹⁾.

The earliest step that is definitely common to many cells entering apoptosis following factor removal is the appearance of oligonucleosome-length fragments of chromatin. This is the result of digestion of the nuclear DNA by endogenous endonucleases, which cut in the linker region where the DNA is most accessible. In BAF3 cells deprived of IL3, it is clear that an event close to the start of DNA fragmentation is the first irreversible step, as IL3 re-addition can rescue cells at this time⁽⁴²⁾. A nuclease stimulated by supraphysiological (mM) levels of calcium has been identified in thymocyte nuclei⁽⁴³⁾, and there is some evidence that this enzyme may be related to the pancreatic digestive enzyme DNase I⁽⁴⁴⁾. However, IL3-dependent cells which apoptose do not express this activity⁽⁴⁵⁾, suggesting the existence of some cell specificity in the nucleases involved in apoptosis. There is also conflicting data regarding the role of endonuclease activation in the onset of the apoptotic pathway. Experiments using millimolar levels of zinc, an inhibitor of the calcium-stimulated nuclease, show either inhibition⁽⁴³⁾ or no inhibition⁽⁴⁶⁾ of cell death. Another nuclease inhibitor, aurintricarboxylic acid, can inhibit cell death, for example in neuronal cells deprived of NGF⁽²²⁾. These inhibitors are rather non-specific, which may explain the different results.

Fig. 2. Stimulation by growth factors of cell survival and proliferation.

Growth factor addition - the choice between proliferation and survival

An overview of the events that follow growth factor addition to cells is presented in Fig. 2 and reviewed in⁽⁴⁷⁾. Unlike growth factor deprivation, it is clear that growth factor addition requires new gene expression and protein synthesis in order to stimulate cell proliferation. Consideration of the pathways by which peptide growth factors can stimulate cell proliferation is beyond the scope of this review; what will be summarised are the mechanisms that may control whether a growth factor stimulates proliferation, or promotes cell survival, in a given target cell. Firstly, it is possible that some factors may trigger specific signalling pathways which stimulate cell survival rather than cell proliferation. For example, insulin-like growth factor I (IGF-I) promotes survival, but not proliferation, of glial cells⁽¹⁶⁾. IGF-I also promotes survival of bone marrow-derived IL3-dependent cell lines and primary cultures, but stimulates much less rapid proliferation than IL3⁽⁴⁸⁾. In these cells interleukin 4 (IL4) also promotes survival but not rapid proliferation⁽⁴⁵⁾. Thus the different rapid signals triggered by IGF-1⁽⁴⁹⁾ or IL4⁽⁵⁰⁾, compared to those stimulated by IL3⁽⁵⁰⁾, may favour survival rather than proliferation. IL3 itself stimulates survival but not maximal proliferation when added in the presence of rapamycin⁽⁵¹⁾ which inhibits p70 S6 kinase activation, suggesting that some of the multiple signalling pathways stimulated by IL3 promote survival rather than proliferation. Furthermore,

analysis of receptor isoforms suggested that different regions of the cytoplasmic domain of the erythropoietin receptor are coupled to proliferation and survival pathways⁽⁵²⁾.

The level of growth factor receptor occupancy can also control the decision of a cell to survive or proliferate in the presence of a given growth factor. Fig. 3 shows that the choice between survival and proliferation by an IL3-dependent cell line, BAF3, can be regulated by the level of IL3 supplied. Low concentrations of IL3 can maintain cell viability, while failing to stimulate cell proliferation. To demonstrate that this choice was controlled by the level of receptor occupancy, the behaviour of 3 cell transfectants expressing intermediate (B8), low (B18), or very low (B3) levels of the interleukin 2 (IL2) receptor β chain was examined. Parental BAF3 cells fail to survive or proliferate in IL2, while transfectants expressing high levels of IL2 receptor β chain (600 intermediate affinity receptors/cell) survive and proliferate as well in IL2 as in IL3⁽⁵³⁾. High concentrations of IL2 can maintain survival, but stimulate very little proliferation in cells expressing sub-optimal levels of receptor (Fig. 3). These data imply that the choice between cell survival and proliferation may be regulated both by the level of factor available and by the level of receptor expressed.

Some differentiated cells are unable to divide, but still require survival factors to inhibit apoptosis. These include neuronal cells which require NGF⁽¹⁸⁾ and eosinophils which die in the absence of interleukin 5 (IL5)⁽⁵⁴⁾. Immortal cells in culture, such as fibroblasts, can arrest and survive in the absence of growth factors. However, whether all non-transformed cells, if they are able to arrest their division, still require survival factors for their maintenance remains an open question.

Positive signalling of apoptosis

Glucocorticoids are potent immunosuppressive hormones produced by the adrenal cortex in response to adrenalin; they therefore provide a link between the nervous and immune systems. It was first demonstrated by Wyllie⁽⁵⁵⁾ that treatment of lymphoid cells with glucocorticoids induced a cell death programme involving the fragmentation of cell chromatin into oligonucleosomes by an endogenous endonuclease (Fig. 4). This induction of apoptosis requires the regions of the glucocorticoid receptor that are known to be required for transactivation of gene expression⁽⁵⁶⁾ and is blocked by inhibition of protein synthesis, implying that *de novo* gene expression is required. This induction of apoptosis probably explains the efficacy of glucocorticoids in the treatment of leukaemia⁽⁵⁷⁾. Many other cancer therapy regimes, including DNA damaging drugs (see for example ref. 8) and inhibitors of nucleotide metabolism⁽⁵⁸⁾, also induce apoptosis in the target tumour cells. Like glucocorticoids, DNA damaging agents also require protein synthesis to stimulate apoptosis⁽⁴²⁾. The stimulation of apoptosis in T lymphocytes by glucocorticoids can be inhibited by the growth factor IL2⁽⁵⁹⁾, or by over-expression of *bcl-2*⁽³⁴⁾. Likewise the stimulation of apoptosis by DNA damaging agents can be blocked by *bcl-2* over-expression in a variety of cells^(34,38,42) and by IL3 in bone marrow cells⁽⁴²⁾. p53 is probably involved in the induc-

Fig. 3. Receptor occupancy controls the choice between survival and proliferation. Clones of BAF3 cells expressing various levels of IL2 receptor β chain were prepared as previously described⁽⁵³⁾ using a recombinant retrovirus encoding human IL2 receptor β chain. Clone B8 expresses 82 intermediate affinity IL2 receptors/cell, clone B18 expresses 11 receptors/cell and clone B3 < 5 receptors/cell. The three clones respond identically to parental BAF3 cells in IL3; the response of clone B8 to IL3-containing conditioned medium (CM)⁽⁵¹⁾ is shown. The response of the three clones to recombinant human IL2, at the concentration shown, is depicted.

tion of apoptosis following DNA damage, as p53 protein is induced upon exposure of cells to DNA damaging agents and mice homozygously deleted for the p53 gene are less sensitive to DNA damage-induced apoptosis⁽⁶⁰⁾.

Positive triggering of apoptosis has also been extensively studied in the thymus, where immature T cells which recognise self antigens, and are therefore harmful, are eliminated. Apoptosis can be stimulated in thymocytes by addition of antibodies which cross-link antigen receptors, or by addition of antigen to thymocytes in organ culture or *in vivo*^(61,62). It is blocked by inhibition of protein synthesis⁽⁴³⁾, by *bcl-2* overexpression⁽³⁴⁾ and by the growth factors IL1⁽⁶³⁾ and IL2⁽⁶⁴⁾. Apoptosis in thymocytes is also inhibited by stimulation of protein kinase C, but stimulated by agents which elevate cyclic AMP or calcium. Why thymocyte antigen receptor triggering leads to apoptosis, whereas mature T cell antigen receptor triggering leads to proliferation, remains unclear. The fact that such cells can be rescued by growth factors suggests that they are analogous to the 'partially activated' fibroblasts which constitutively express c-myc, and that insufficient growth factors are produced when cells are stimulated in the thymic micro-environment or in culture to rescue activated cells (Fig. 4). Indeed inhibition of c-myc expression can prevent apoptosis induced by antigen receptor triggering in a T cell hybridoma⁽⁶⁵⁾. Mature T lymphocytes can also be induced to apoptose if triggering of the auxiliary surface molecule CD4 is uncoupled from triggering of

the antigen receptor. This may explain some of the cell death by apoptosis seen in lymphocytes of human immunodeficiency virus type 1 (HIV-1)-infected individuals, as the HIV-1 envelope can trigger apoptosis by binding to CD4⁽⁶⁶⁾.

Finally, there is at least one mechanism of apoptosis induction which is not known to be inhibited by growth factors or by *bcl-2* overexpression (see Fig. 4). Cytotoxic T lymphocytes induce apoptosis in their target cells (reviewed in ref. 67). A nuclease in the target cell is responsible for chromatin fragmentation, though this is not essential for cell

death⁽⁶⁸⁾. This process is not inhibited by *bcl-2* over-expression⁽⁶⁹⁾.

Triggering of the murine cell surface antigen Fas and its human homologue APO-1 also induces apoptosis. Mice defective in Fas are unable to remove self-reactive thymocytes by apoptosis and therefore suffer from auto-immune disorders⁽⁷⁰⁾. Fas has also been implicated in the induction of apoptosis by cytotoxic T lymphocytes⁽⁷¹⁾. Antibodies to APO-1 induce apoptosis in some cells⁽⁷²⁾, but can also stimulate proliferation in others. This may imply that Fas/APO-1 can act a little like the T cell antigen receptor and stimulate either proliferation or apoptosis. Likewise, tumour necrosis factor- α (TNF- α) can stimulate either apoptosis (reviewed in ref. 67) or proliferation⁽⁷³⁾ of target cells. The cellular receptor for TNF- α is homologous to Fas/APO-1 and it may therefore be possible that they generate similar second messengers which lead to apoptosis in some cells. Indeed, addition of ceramide, a sphingolipid precursor generated by TNF- α receptor triggering, can induce apoptosis in lymphoid cells⁽⁷⁴⁾. Although it is not completely clear, induction of apoptosis by TNF- α or anti-APO-1 may also be inhibited by growth factors or Bcl-2, since it has been shown that TNF- α -induced apoptosis can be inhibited by the survival factor CNTF in glial cells⁽⁷⁵⁾ and APO-1-induced apoptosis may be inhibited by *bcl-2* expression. This raises the interesting possibility that growth factors may also play an inhibitory role in almost all situations in which apoptosis is positively stimulated, and suggests that regulation of growth factor levels is not only of paramount importance in the control of cell proliferation but also in maintaining viability of cells susceptible to undergoing apoptosis.

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Mary K. L. Collins and Gordon R. Perkins are at the Chester Beatty Laboratories, Institute of Cancer Research, Fulham, London, UK. Gemma Rodriguez-Tarduchy is at the Instituto de Investigaciones Biomédicas CSIC, Madrid, Spain, and María Angela Nieto is at the Instituto Cajal CSIC, Madrid, Spain. Abelardo López-Rivas* is at the Instituto de Parasitología y Biomedicina CSIC, c/ Ventanilla 11, 18001 Granada, Spain.

*Author for correspondence.