Links between apoptosis, proliferation and the cell cycle

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Accepted: 11 April 2004

ABSTRACT

Many physiological processes, including proper tissue development and homeostasis, require a balance between apoptosis and cell proliferation. All somatic cells proliferate via a mitotic process determined by progression through the cell cycle. Apoptosis (programmed cell death) occurs in a wide variety of physiological settings, where its role is to remove harmful, damaged or unwanted cells. Apoptosis and cell proliferation are linked by cell-cycle regulators and apoptotic stimuli that affect both processes. This review covers recent developments in the field and examines new evidence of the interconnection between apoptosis and cell proliferation.

KEY WORDS: Apoptosis. Cell cycle. Proliferation.

Introduction

The cell cycle is divided into four phases, and the cellular decision to initiate mitosis or to become quiescent (G_0 state) occurs during the G_1 phase. Oncogenes have a dual role: they can induce both proliferation and apoptosis (Fig. 1).

As somatic cells proliferate, the cell-cycle progression is regulated by positive and negative signals. Apoptosis and mitosis share common morphological features such as cell shrinkage, chromatin condensation and membrane blebbing. Additionally, cell-cycle genes such as *p53*, *RB* and *E2F* have been shown to participate in both the cell cycle and in apoptosis. Thus, the balance between apoptosis and proliferation must be strictly maintained to sustain tissue homeostasis (Fig. 2).

The link between apoptosis and proliferation is suggested by studies that have demonstrated the presence of large numbers of dying cells in proliferating cell populations *in vivo*. Here, the current view of the interactions that take place between apoptosis, the cell cycle and proliferation is discussed.

Are proliferation and apoptosis linked?

Cell proliferation, differentiation and death are fundamental processes in multicellular organisms, and several lines of evidence link apoptosis to proliferation. Firstly, uncontrolled proliferation can be associated with a high level of apoptosis. A number of dominant oncogenes (e.g., *c-Myc*) appear to induce apoptosis, which suggests that the cell proliferation and apoptosis pathways are closely linked.[1]

Reid *et al*.[2] demonstrated that myeloid progenitors derived from the bone marrow of $CCR^{-/-}$ mice (ligand for MCP-1 chemokine) show an increased cycling rate and enhanced apoptosis. Traver *et al*.[3] showed that expression of the myeloid activation marker Mac-1 correlates with Fas expression levels. They also showed that exposure to granulocyte macrophage-colony stimulating factor (GM-CSF) and interleukin (IL)-3 increased Fas expression on myeloid progenitor cells. These examples infer a positive relationship between apoptosis and proliferation.

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However, an inverse relationship was proposed by Koury,[4] who suggested that when over-production of progenitor cells occurs, the excess undergo apoptosis. This means that an increase in the level of demand could be met by a reduction in apoptosis. In this case, proliferation may remain constant while the rate of apoptosis changes; thus, there is no strict relationship between proliferation and apoptosis. This hypothesis is supported by radiolabelled iron ferrokinetic studies in hypertransfused mice that showed continued production of erythroid progenitor cells but no increase in their number, indicating the direct involvement of apoptosis in this situation.[5]

As both hypotheses involve changes in the level of proliferation and apoptosis, the difference between them may be only a matter of degree. A final possibility that must be considered is that apoptosis in haemopoietic progenitor cell populations is linked to pathology or abnormality such as growth factor deprivation or oncogene expression.

Cell cycle-related proteins and apoptosis

The molecules that regulate cell-cycle progression are well defined. Meikrantz and Schlegel[6] demonstrated that the cell cycle and apoptosis may be linked, and provided supplied arguments to support such a link. First, apoptosis is almost present in proliferating cells. Second, molecules acting on cells in late G^1 phase are also required for apoptosis. Third, passage of a cell from late G^1 to the S phase of the cell cycle is controlled by p53 and cdk. Finally, artificial manipulation of the cell cycle (e.g., retroviral transduction) could either abolish or potentiate apoptosis.

Furuya *et al*.[7] showed that apoptosis can occur at any phase of the cell cycle, as the metabolic machinery necessary is present throughout. Furthermore, they demonstrated that

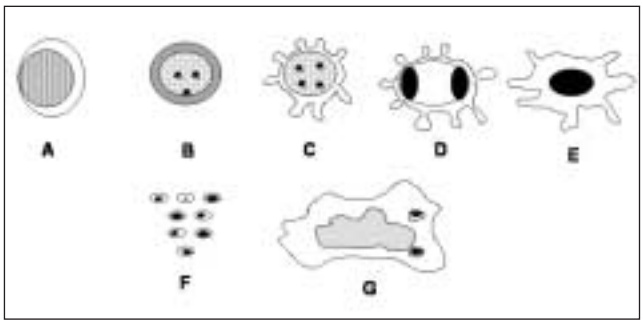

Fig. 1. Apoptosis in sequence: A) normal resting cell; B) cell volume is lost and chromatin clumped; C) blebbing process; D) chromatin collapsed to the margins of the nuclear envelope; E) nucleus clumped into a black hole; F) cell breaks down into apoptotic bodies; and G) apoptotic bodies ingested by macrophage.

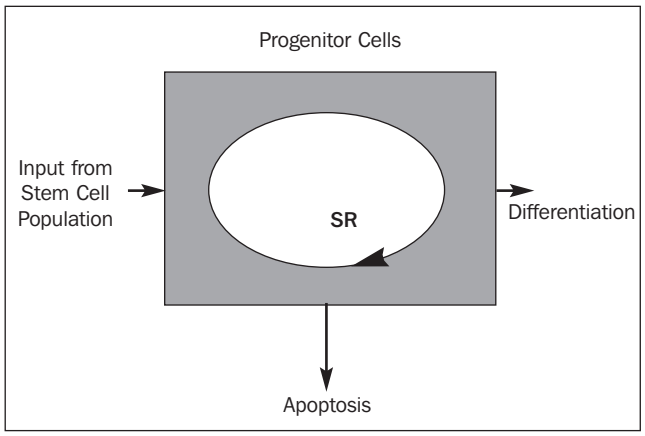

Fig. 2. Progenitor cell population: gain is produced by input from the stem cell population: loss occurs through differentiation and apoptosis.

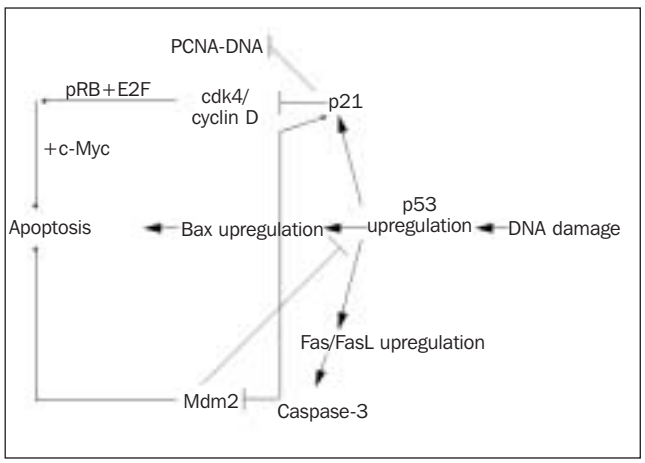

Fig. 3. Schematic representation of the negative feedback of caspases.

the treatment of both human and rat androgen-independent prostate cancer cells with thapsigargin (TG) caused the release of Ca^{2+} from intracellular stores (e.g., endoplasmic reticulum [ER]). Constant depletion of Ca^{2+} from ER resulted in a concomitant influx of extracellular Ca^{2+} into the cell. After 24-hours, cessation of cell-cycle progression occurred and cells were arrested in G_0. At this stage, morphological changes associated with apoptosis were visible. These results indicate that elevation of Ca^{2+} (intracellular free Ca^{2+}) can initiate apoptosis.

A number of studies have shown that cell-cycle regulators could interconnect with proliferation and apoptosis. Both *p16*$^{-/-}$ and *p21*$^{-/-}$ mice are deficient in key cell cycle genes, while lpr and gld mice (Fas and FasL mutant mice, respectively) have a defective apoptotic mechanism. However, Lewis *et al*.[8] showed that *p16*$^{-/-}$ knockout mice have a higher self-replication capacity than do wild-type (WT) mice, which links the cell cycle and apoptosis. Similarly, *p21*$^{-/-}$ knockout mice have a higher self-replication capacity than do WT mice.

Recent work[9] showed that both lpr and gld mice have a higher self-replication capacity than do WT mice, which links apoptosis and proliferation. Thus, it can be inferred that the presence of fully functional genes that regulate both the cell cycle and apoptosis will maintain the balance between the rate of cell division and apoptosis in any population *in vivo*. Therefore, malfunction in, or loss of, a ny of these genes may lead to an increase in their self-replication.

Miyashita *et al*.[10] showed that the restoration of *p53* function resulted in down-regulation of Bcl-2 levels and the occurrence of apoptosis. They also showed that *p53* activates the Bax promoter and induces high levels of Bax messenger RNA (mRNA) and protein. Moreover, Yin *et al*.[11] showed that Bax is required for 50% of p53-induced apoptosis.

Gomez *et al*.[12] demonstrated a relationship between *p27*, *cdk2* and apoptosis in thymocytes, which was modulated by *p53*, *Bcl-2* and *Bax*. Thus, *cdk2* activation seems to be the key point at which the cell cycle and apoptosis meet.

Janicke *et al*.[13] showed that the retinoblastoma (*RB*) gene is cleaved during apoptosis, at the caspase consensus cleavage site (DEAD), resulting in a protein product of 50 kDa. Dou *et al*.[14] showed that RB is also cleaved on an interior site, producing proteins of 48 and 68 kDa. Fattman *et al*.[15] demonstrated that caspase-3 and caspase-7 cleave RB at the DSID cleavage site, resulting in proteins of 68 and 48 kDa. These findings support a two-step model for RB cleavage and a promoting role in chemotherapy-mediated apoptosis.

Browne *et al*.[16] demonstrated that RB is cleaved at the carboxyl terminal, producing 43- and 30-kDa protein fragments. In addition, ZVAD was found to inhibit the cleavage of RB, poly-ADP-ribose polymerase (PARP) and apoptosis. In contrast, YVAD did not inhibit primary carboxyl terminal cleavage of RB and PARP. These results suggest that different caspases are responsible for the cleavage of different substrates during apoptosis.

Accumulating evidence suggests that p16 and p21 are involved in the activation of caspases. Chen and colleagues[17] suggested that treatment of cancer cells with chemotherapeutic agents causes up-regulation of *p21*, which is associated with the activation of caspase-3 and -9. Chemotherapeutic treatment leads to the phosphorylation of p21, followed by the phosphorylation of Bcl-2, resulting in a reduction in the dimerisation complex of Bcl-2 and Bax, where Bax becomes free. Cytochrome-C is then released, causing caspase-3 activation and apoptosis.

Snoeck *et al*.[18] showed that treatment of papillomatous lesions with Cidofair leads not only to the up-regulation of *RB* and *p21* but also to the induction of apoptosis. Fukuoka *et al*.[19] showed that ectopic p16 expression increased the sensitivity of non-small-cell lung carcinoma to the

chemotherapeutic agent CPT-11. This was accompanied by caspase activation and enhanced apoptosis. In contrast, Suzuki and colleagues[20] demonstrated that survivin interacts with cdk4, and, as a result, p21 is released from its complex with cdk4 and interacts with Pro-caspase-3 in mitochondria, resulting in inhibition of apoptosis.

Cell-cycle transitions are mediated through multiple phosphorylations of cyclin-cdk complexes. RB phosphorylation releases E2F transcription factor, which activates certain genes during S phase. Activation of p21 results in negative regulation of the cell cycle. p21 interacts with cdk and proliferating cell nuclear antigen (PCNA), resulting in a block on DNA replication. *p21*$^{-/-}$mice are also unable to stop the cell cycle in G_1 in response to DNA damage. As with p21, p27 inhibits cyclin E and A binding to cdk2, and both p21 and p27 are absent from non-proliferating cells.

A further relevant interaction in the regulation of apoptosis is inhibition of p53 function mediated by Mdm-2, which is cleaved by caspase-3. This implies the existence of an autoregulatory loop during p53-induced apoptosis. Activation of p53 potentially can amplify p53 apoptotic signalling in the cell by stimulating caspase-3-dependent cleavage of Mdm-2. Consistent with this interpretation, Bcl-2 can also increase Mdm-2 activity and inhibit p53-induced apoptosis (Fig. 3).

Apoptosis-related proteins and the cell cycle

Several studies indicate that apoptosis-regulating proteins can have an impact on the cycle. O'Reilly *et al*.[21] demonstrated that the expression of Bcl-2 or Bcl-xL reduces the rate of cell division and the turnover of thymocytes *in vivo*. Similarly, Strasser *et al*.[22] showed that Bcl-2 and Bcl-xL inhibit apoptosis of proliferating cells. The surviving cells undergo cell arrest and accumulate in the G_0/G_1 phase of the cell cycle.

Brady and colleagues[23] demonstrated that the over-expression of Bax and Bcl-2 in T cells of transgenic mice resulted in perturbations in the dividing thymocytes. Bax was found to increase the number of cycling thymocytes, but Bcl-2 had the opposite effect. In activated T cells, they found that Bcl-2 over-expression delayed p27degradation, whereas Bax accelerated it.

Domen *et al*.[24] produced *Bcl-2* transgenic mice that over-expressed *Bcl-2*. They found that haemopoietic stem cells (HSC) from WT mice died after growth factor withdrawal, whereas HSC from *Bcl-2* transgenic mice remained viable. More importantly, HSC from *Bcl-2* transgenic mice proliferated more rapidly and extensively (in the presence of a cocktail factors including IL-1, IL-3, IL-6, SCF, Flt3-ligand) than those of WT mice. In addition, there was a delay in cell-cycle entry.

The most dramatic difference between WT and *Bcl-2* transgenic mice was revealed when HSCs were cultured in the presence of SCF. Only 20% of WT HSC remained viable after one week, whereas HSC from Bcl-2 transgenic mice showed enhanced survival and more vigorous proliferation. Bcl-2 over-expression and SCF/c-kit signalling was found to be sufficient for HSC proliferation, although it should be noted that proliferation also resulted in differentiation of myeloid progenitor cells.[24-25]

Furthermore, at least three pieces of evidence demonstrate that caspase-3 is capable of cleaving p16, p21 and RB. Kim *et al*.[26] showed that caspase-3 cleaves p21, p27 and PARP via induction of cell-cycle arrest in cells activated for 48 hours by tumour growth factor (TGF)-β1 treatment. They also suggested that caspase-3 activation by TGF-β1 may initiate the conversion of cell-cycle arrest to apoptosis. Katsuda *et al*.[27] demonstrated that induction of p16 (Adv/p16) triggers apoptosis in a p53-dependent manner, which is associated with a reduction in Bcl-2 expression. Caspase-3 activation occurred after five days of Adv/p16 induction. RB was also cleaved, probably by caspase-7, leading to apoptosis. Gervais *et al*.[28] showed that the ectopic induction of p53 expression leads to p21 cleavage, mediated by caspase-3, and apoptosis. It is thought that p21 cleavage might affect its potential inhibitory effect in the cell cycle, resulting in the failure of cell-cycle arrest and the inhibition of binding to PCNA (reviewed[29,30]).

Conclusions

From the evidence presented here, three statements can be made. First, apoptosis is not restricted to a particular part of the cell cycle. Second, the dual hypothesis (i.e., the linkage between proliferation and apoptosis) has major role in neoplasia. Third, the regulation of the cell cycle is coupled to cell death and has major significance in cell turnover and tumourigenesis.

Improved understanding of apoptosis genes has introduced an entirely new modality for treating cancers. Although these anti-apoptotic mechanisms are currently obscure, many of these new developments will be implemented rapidly and will soon play an important role in chemotherapeutic strategies in the treatment of cancer. Such strategies are likely to radically change the management of patients with this disease.

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