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Review paper

Programmed cell death (apoptosis) and response to anti-cancer drugs

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Programmed cell death (apoptosis) is a conserved, natural mechanism for the removal of redundant and unwanted cells during normal development. This article reviews the evidence that apoptosis may also control the response of tumor cells to treatment with cytostatic drugs. Whereas most clinically used anti-cancer drugs can activate late events of apoptosis (DNA degradation and morphological changes) there are differences in essential signalling pathways between pharmological cell death and the physiological induction of an active suicide programme. However, deregulation of normally integrated cell cycle progression appears a central signalling event in most forms of apoptosis, linking cell cycle control, DNA repair and cell death. Whether apoptosis is the cause or the consequence of drug-induced cell death remains to be established.

Key words: Apoptosis, cell cycle, cytostatic drugs, programmed cell death.

Introduction

Treatment of tumor cells with cytostatic drugs or with ionizing radiation usually results in DNA damage. The subsequent decision of the treated cell whether 'to die or not to die' is the outcome of a complex balance of promoting and protecting factors, in which the capacity of DNA repair plays a crucial role. There is, however, an increasing awareness that more distal events in drug-mediated killing of cells can also be decisive for the outcome of treatment. The ability to mount a programmed cell death (apoptosis) attracts much attention as a possible discriminator between survival and death. The significance of apoptosis in normal development and tumor growth has been the subject of numerous papers and many reviews. Many cytostatic drugs with widely different modes of action can also induce apoptosis and this has led to the speculation that cell death is not primarily caused by the effect of a drug on its cellular target, but that various drug–target interactions function as a signal for a conserved, adaptive response to damage: apoptosis. Recent discoveries on the (onco) genetic control of apoptosis and its intimate association with cell cycle regulation and DNA repair have raised great hopes for a better understanding of the intrinsic sensitivity of cells to cytostatic drugs, if not for the development of entirely new strategies of anti-cancer treatment.

Apoptosis in normal development

Programmed cell death (apoptosis) has been known for many years to be an important regulator in fundamental developmental processes that include embryogenesis, tissue involution and clonal selection in the immune system.[1–3] Apoptosis is an active process accompanied by distinct morphological and biochemical changes that include internucleosomal DNA degradation by endonucleases and nuclear condensation. It can be distinguished from necrotic death which proceeds by early loss of membrane integrity and collapse of cellular homeostasis.[4] Apoptosis is triggered by a variety of positively and negatively acting natural signals such as natural killer cell attack,[5] withdrawal of growth factors[6] and binding of antibodies to specific surface receptors.[7,8] The sequence of events leading to apoptosis is not yet completely understood. Most information stems from the suicide programme induced in thymocytes cells by glucocorticoid hormones and other signals,[4] as illustrated in Figure 1.

Accumulating evidence supports the notion that apoptosis is under (onco)genetic control. In *Caenorhabditis elegans*, the *ced* genes are positive or negative regulators of apoptosis, acting in concert with a series of genes involved in programmed cell death during normal development.[9] In mammalian cells, genetic control of apoptosis is most

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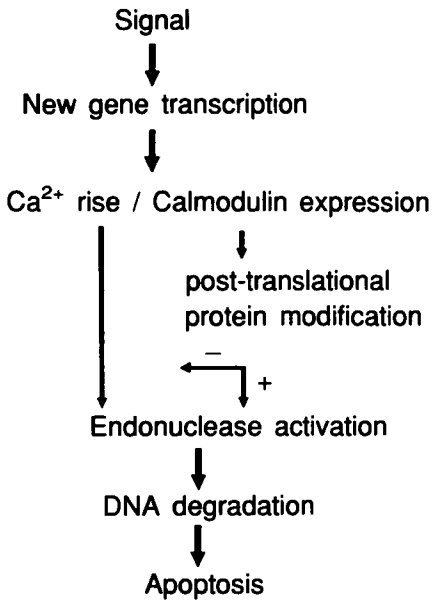

Figure 1. Pathways in apoptosis. The main steps in apoptosis induced in thymocytes by glucocorticoid hormones and other signals. In the case of cytostatic drugs, only DNA degradation and morphological changes have been consistently observed. Drugs affecting protein phosporylation or dephosphorylation can modulate the apoptotic response.

convincingly demonstrated by the proto-oncogene *bcl*-2, representing a novel class of genes that control cell death by suppressing apoptosis[10–12] and by the wild-type tumor suppressor gene p53 that induces apoptosis in myeloid leukemic cells[13] and in sarcoma cells with unrepaired DNA damage.[14,15]

Apoptosis and anti-cancer treatment

There is mounting evidence that failure of apoptosis in tumor cells may contribute to the evasion from physiological controls and resistance to immunological defenses.[16] Accordingly, the ability of cancer cells to mount a programmed death may be an important arbiter of therapeutic response.[17] The importance of apoptosis in the treatment of malignant disease has long been recognized in the effects of hormonal ablation in hormone-dependent tumors. The lympholytic effect of glucocorticoids in acute lymphocytic leukemia is another example of the therapeutic potential of induction of apoptosis in selected malignant diseases. Triggering of apoptosis by monoclonal Fas/APO 1 antibody[7] or by somatostatin analogs[18] are more recent applications of this principle.

In addition, apoptosis can be activated by many types of cytostatic drugs[19,20] or ionizing radiation[21] and notably so in leukemia/lymphoma cells. Moreover, blocked apoptosis by overexpressed *bcl*-2 appears to protect against a range of cytotoxic drugs and ionizing radiation.[22,23] All this supports the general concept that apoptosis is a major pathway of cell death by cytostatic drugs.

Programmed and unprogrammed cell death

The almost ubiquitous finding of apoptosis as a terminal event in the action of cytostatic agents and the notion that cell death is internally programmed and under genetic control, suggest that the intrinsic sensitivity of tumor cells to cytostatic treatment is determined by the probability to engage an apoptotic programme. On closer investigation, however, several theoretical considerations and experimental observations question this tenet.

Morphological changes and the formation of 'DNA ladders' by endonuclease action are frequently used as the hallmarks of apoptosis. The sequence of events leading to cell death by cisplatin in fibroblasts[24,25] indicates that irreversible G_2 arrest and cell detachment precede typical DNA degradation. Accordingly, the formation of DNA ladders on agarose gels and morphological changes characteristically associated with apoptosis are not necessarily early and causative events in cell death from which cells can be rescued.

Induction of (late) phenomena of apoptosis by drugs may differ on essential points from programmed cell death by natural triggers. Most drugs induce DNA damage and apoptosis in actively cycling cells,[26,27] and the commonly held view is that the affected cells die in a mitotic catastrophy. In contrast, many 'natural' triggers for programmed cell death, including glucocorticoid hormones, act at G_1–G_0 transition[28] and cells die in a process conveniently described as 'premature aging'. Apoptosis in thymocytes by glucocorticoid hormones is interrupted by inhibition of protein and RNA synthesis, and involves calcium fluxes and *de novo* synthesis of endonuclease and other unidentified proteins.[29] While a matter of dispute in glucocorticoid-mediated lysis of leukemic cells,[30–33] drug-induced killing of hematopoietic cells is clearly insensitive to inhibition of RNA or protein synthesis, and does not involve Ca^{+2} fluxes and new gene expression.[26] It has been argued therefore that in cells rapidly responding to cytostatic drugs, e.g. those of hematopoietic origin, the suicide programme is con-

stitutively activated, requiring only the release from an unknown inhibitory factor.[33] Thus, the route to death by physiological apoptosis appears to differ from pharmacological cell kill in important signalling steps and checkpoints, sharing only distal events. Indeed, killing by cytostatic drugs can be viewed as unprogrammed cell death, related to programmed cell death as murder is to suicide.[25]

In cancer chemotherapy and radiotherapy, irreversible loss of clonogenic capacity is critical for cure. While apoptosis is obviously an irreversible route to death, it is not an exclusive one. Cells that are protected from apoptosis may yet have lost clonogenic capacity and die later in failing mitosis or become sterilized, but remain viable. In other words, their eventual death is postponed, not prevented. In tissue culture, irradiated cells may go through a few divisions, but still be defined as 'killed' because of loss of reproductive capacity.[34]

However, the evidence to the contrary should be weighed against those few reports which have established a correlation between modulation of apoptosis and altered sensitivity to cytostatic drugs or ionizing irradiation. Overexpression of the deregulated *bcl*-2 gene in human leukemic cells simultaneously inhibits apoptosis and promotes survival in clonogenic assays after exposure to some (but not all) cytostatic drugs, albeit at a very low plating efficiency.[35] Drugs acting on kinases and phosphatases can concomittantly promote or inhibit apoptosis and clonogenic cell survival.[36,37] It is difficult, however, to conclude from these observations that the modulation of apoptosis was the direct cause of altered clonogenic survival. Drug sensitivity may have changed as a consequence of indirect effects of the BCL-2 protein or of the inhibitors, e.g. by altered cell cycle kinetics, changes in drug transport and metabolism or by modulation of repair capacity.

In order to appreciate better the similarities and differences between drug-induced cell kill and physiological programmed cell death, it is important to consider how damage from cytostatic drugs is sensed. What is recognized and where is the input in the sequence of events leading to death, as depicted in Figure 1?

Cell proliferation and apoptosis

Spontaneous apoptosis of quiescent hematopoietic cells is antagonized by mitogenic growth factors whose function can be substituted by overexpressed *bcl*-2.[6,11,38] Likewise, the activation of apoptosis by glucocorticoids in lymphoid cells is preceded by growth inhibition[28] and can be inhibited by growth factors[39] or the deregulated *bcl*-2 gene.[22,23,40] All this is consistent with the view that induction of apoptosis by physiological signals and glucocorticoids, and thus the protection by *bcl*-2, can be positioned at the G_1–G_0 transition, i.e. near to a point-of-no-return for mitogen-induced rescue.[2]

In contrast, cytostatic drugs act mainly on actively cycling cell, and cell cycle progression is often required for the manifestation of cell death. Depending on the type of drug, the killing of proliferating cells is initiated in different phases of the cell cycle, most often involving transient cell cycle arrest in that phase but eventually culminating in abortive mitosis. Proliferating cells exposed to cytostatic drugs thus appear fully competent in mounting at least the final steps of apoptosis, without an initial setting of the cells to a G_1–G_0-like phase. Finally, apoptosis in mature T cells induced by crosslinking of surface receptors (hyperactivation) appears to be dependent on active proliferation.[41] These conflicting observations, namely that apoptosis requires either growth inhibition at G_1–G_0 or blockade in specific cell subphases, or even active proliferation, may suggest that its induction is completely unrelated to proliferation. Alternatively, common cell cycle events may be involved in each of the above-described phenomena.

Deregulated cell cycle control

One of the most exciting developments in cell biology is the rapidly growing insight that cell cycle progression is under tight genetic control. The mammalian cell cycle is conveniently described as being composed of two cycles: the DNA replication cycle and the cell division cycle (Figure 2). Physiological uncoupling of these two cycles occurs during haploidization of meiotic cells and endoploidization of liver cells or megakaryocytes. The two cycles are driven and coordinated by oscillations of the various members of the cyclin family whose gene products 'mix and match' with cyclin-dependent protein kinases (CDKs), the mammalian homologues of yeast *cdc*2 gene products.[42] The DNA replication and the cell division cycle are tightly coupled at multiple checkpoints. To ensure fidelity in DNA replication and in cell division, completeness of any cyclic process is controlled first before initiation of the next step.

The role of growth factors at the G_0–G_1 boundary, affording mitogenic rescue from aging and differ-

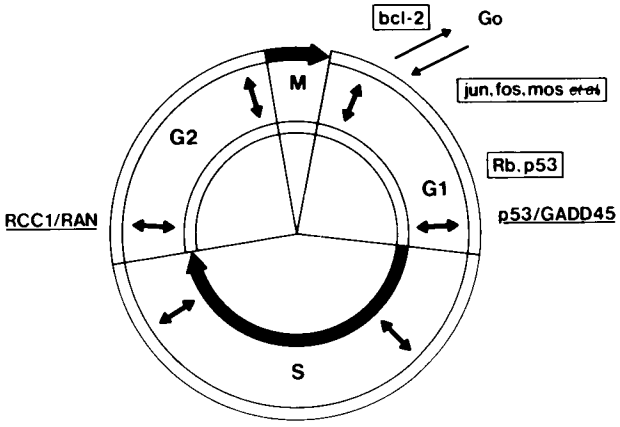

Figure 2. Cell cycle control. The mammalian cell cycle is composed of the DNA replication cycle (inner) and the cell division cycle (outer). Both cycles are integrated at multiple sites by cyclin/*cdc2*(CDK) interactions, indicated by black double arrows).[42] Oncogenes substituting for growth factors (e.g. *jun*, *fos*, *mos*)[43] or promoting cell survival (*bcl*-2),[47,48] and tumor-suppressor genes monitoring progression through G_1 phase[44] are indicated in small boxes. Examples of gene interactions involved in the control of DNA structure at G_1–S [14] and S–G_2 [45] transition, respectively, are underlined.

entiation, is of recognized importance for the commitment to proliferation, as is their substitution by various oncogenes.[43] Programming of cell proliferation and differentiation is also under control of wild-type alleles of tumor suppressor genes.[44] In BHK cells the RCC1 gene (repressor of chromatin condensation) is probably implicated in the control of DNA replication and thus couples completion of S phase with initiation of mitosis.[45] Likewise, at the end of G_1 phase, the DNA is monitored for possible damage before the start of DNA duplication. Detection of DNA damage stabilizes the wild-type p53 protein, halting the cells at the G_1–S transition and enabling the repair of DNA damage. If this fails, cells with unrepaired DNA are directed into an apoptotic pathway.[14,15] The normal development of p53 knock-out animals indicates that suppression of mutation rather than of proliferation is the main function of this tumor suppressor gene. Genes involved in these regulations are being discovered with unprecedented speed, and the results of these investigations will soon allow an integrated view on such widely diverse phenomena as cell cycle control, DNA repair and programmed cell death.

It appears that any disruption of normally integrated cell cycle events can act as a trigger for apoptosis, and that cell cycle genes may function as regulators of this process. Unscheduled expression of the oncogene c-*myc* causes apoptosis in various cell systems by generating inappropriate mitogenic signals.[46–48] Cytostatic drugs have widely different modes of action, yet a common sequela of most anti-cancer drugs is to disrupt coordinated cell cycle progression. For example, during inhibition of DNA synthesis by anti-metabolites, protein content and cell volume continue to increase to G_2 levels. The observation by Kung *et al.*[49] that cell death by agents that block the cell division cycle in mitosis or the DNA replication cycle in S phase is prevented by simultaneous inhibition of protein synthesis, is primordial to this view. In fact, reported inhibitions of apoptosis by cycloheximide, cyclosporin or other metabolic inhibitors may well be due to prevention of cell cycle deregulation (i.e. interruption of the stimulus) rather than to interruption of the apoptotic programme itself (the response). Conversely, promotion of cell death by caffeine or 2-aminopurine has been attributed to the overriding of physiological controls on cell cycle regulation.[37] These purine analogues are thought to inhibit *cdc2* protein kinase activity, advancing the cells past checkpoints resulting in premature truncation of repair processes and enhanced cell death. Protection from apoptosis by stimulation of protein phosporylation[36] could represent the reverse action.

In the case of growth factor deprivation or hormonal ablation, cells are also confronted with conflicting or unbalanced mitogenic signals. This is illustrated by the paradoxical observation that negative trophic signals can stimulate the transcription of proliferation-related oncogens[50,51] and can be synergistic with unscheduled c-*myc* expression in initiating apoptosis.[41,46] Finally, it has been proposed recently that programmed cell death of terminally differentiated neuronal cells is essentially a response to aberrant proliferation signals.[52]

The hypothesis that cell cycle deregulation is a general trigger for apoptosis, while distressingly vague, is attractive in offering an explanation of how various and different inputs culminate in a common response. It holds essentially that cell death is activated by natural control processes whose function is to allow repair of low level damage to DNA, but to eliminate those cells in which repair falls short or which are subject to inappropriate mitogenic signals. The counter-intuitive notion of an association between cell cycle progression and cell death yet accounts for the observation that high turnover hematopoietic tissues are particularly prone to apoptosis.[53] Likewise, it is not surprising in this context that events such as crosslinking of T cell receptors, exposure to tumor necrosis factor (TNF)

or increased expression of certain oncogenes can, depending on cell type and developmental stage, either stimulate proliferation or induce apoptosis.[16,52,53]

Concluding remarks

Programmed cell death (apoptosis) is a natural and conserved mechanism for the removal of redundant or unwanted cells during normal development.[1,2,4,52] Several observations suggest that anti-cancer drugs elicit a programmed cell death, either completely or partly, by disrupting normally integrated and genetically controlled cell cycle events.

From a therapeutic point of view, however, the central question is not how a cell eventually dies but whether a failing or delayed apoptotic response confers resistance to anti-cancer treatment. This is conceivably true for treatment modalities that are intrinsically based on the initiation of a full suicide programme, e.g. the lysis of leukemic cells by glucocorticoid hormones, or the involution of hormone-dependent tumors after hormonal ablation. In these cases, modulation of response by interference with the apoptotic programme and the development of novel treatment strategies are basically possible.

As for the majority of tumors and cytostatic treatments, it is still largely speculative that the ability to mount a programmed cell death controls intrinsic drug sensitivity. There is increasing albeit circumstantial evidence to suggest that many forms of drug-induced and physiological cell death are initiated by deregulation of cell cycle integration. However, it is uncertain whether cytostatic drugs elicit a complete suicide programme, subject to genetic controls and amenable to pharmacological interventions. Rather, severe cell cycle disruption by cytostatic drugs can be the primary cause of death,[49] which may or may not be accompanied by the manifestation of distal and irreversible steps of the apoptotic process. The amount of drug-induced damage and the rate with which it is inflicted may direct the route to death. This dilemma is depicted in Figure 3.

The question of cause–effect in drug-induced apoptosis seems to be most crucial in order to appreciate the impact of apoptosis on chemotherapy and to position the protective action of *bcl*-2 and other regulators of death to be discovered in the near future. So far, *bcl*-2 is the only documented

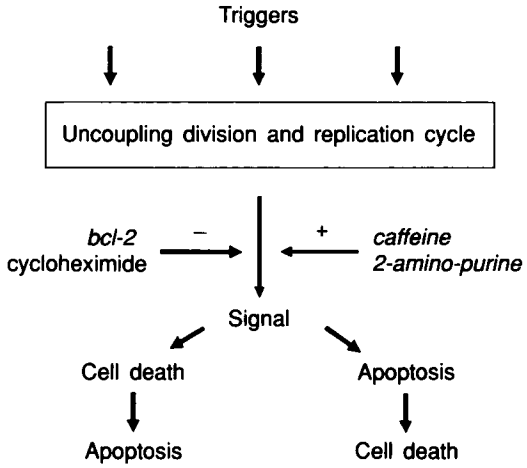

Figure 3. Induction and control of apoptosis. In this speculative scenario, uncoupling of cell cycle integration is a central signalling event for most inducers of apoptosis. The signal can be amplified by drugs that override cell cycle control[37] or mitigated by *bcl-2* or cyclohexamide.[49] In the case of physiological triggers, the resulting signal induces apoptosis leading to cell death (right). Severe damage from drugs can be a primary cause of cell death with apoptosis as a secondary consequence (left).

(negative) regulator of apoptosis, but its action *in vivo* is confined to subsets of lymphoid cells. The observation that deregulated *bcl*-2, in spite of its suggestive effects in tissue culture models, has no clear impact on treatment outcome in clinical lymphoma[54] should be a strong caveat against too high expectations. Some current hypotheses regarding programmed cell death and drug sensitivity are definitely premature and may eventually face their own apoptosis.

Yet, the recent discoveries of the role of p53 in promoting apoptosis suggest quite a different association between apoptosis and the clinical response to chemotherapy and radiotherapy. As discussed above, apoptosis may be an effector but not the controller of the regression of solid tumors or remission-induction of leukemia during chemotherapy with cytostatic drugs. However, failing or delayed apoptosis can increase the probability of relapse by promoting the survival of cells with incompletely repaired DNA damage, giving rise to the emergence of rare, therapy-resistant variants.[15]

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