Pergamon

European Journal of Cancer Vol. 32A, No. 6, pp. 921–926, 1996

Printed in Great Britain
0959-8049/96 $15.00+0.00

S0959-8049(96)00080-9

Apoptosis and Chemotherapy Resistance

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INTRODUCTION: CELL DEATH AS AN ADAPTIVE RESPONSE TO DAMAGE

IN TRYING to understand why many cancer cells do not die after drug treatment, and are drug resistant, it is appropriate to ask why other tumour cells *do* die after the imposition of cellular damage. The determinants of drug sensitivity, of drug resistance and of selectivity have traditionally been defined by the principles laid down by Paul Ehrlich, the "father" of chemotherapy. These suggest that a chemical will have a selectively toxic effect if a target is uniquely expressed in a particular cell type and if enough of the active drug or toxin interacts with it to perturb cellular metabolism. Obviously these are critical requirements for the imposition of pharmacological effects and much of this issue deals with mechanisms whereby they are circumvented. However, cells are not passive recipients of drug-induced perturbations. Instead, toxins initiate cellular responses to the imposition of damage. Perhaps the most important of these responses is cell death.

Cell death, in the context of a multicellular organism, is not an arbitrary and passive event: the normal loss of cells in development and in homeostasis demands that death is controlled. That control is necessary not only for the maintenance of cellular numbers, where cell gain must be balanced by loss, but also to prevent the inflammatory response that would follow rupture of excess or damaged cells. The engagement of a controlled cell death is also an important mechanism for preventing the emergence of mutant and potentially malignant cells: DNA damage, as will be described below, is a potent activator of cell death in cell types where amplification of numbers would lead to the emergence of many mutant progeny. Those tissues in which cells respond readily to delete themselves by an "altruistic" apoptosis are less likely to undergo malignant transformation than those where this response to DNA damage is attenuated. This idea may lie at the heart of an understanding of why the common cancers are resistant to DNA damaging drugs, and is discussed below.

What is critical about the concept of cell death as an "adaptive" response to damage is that it is genetically controlled. The resurgence of interest over the past five years in the phenomenon of cell death was largely stimulated by the discovery of genetic determinants of cellular survival and cell death. The finding that there was conservation of sequence between some of the death regulating genes required for the proper development of the nematode worm *Caenorhabditis elegans* and those of man [1] was particularly exciting, not least because it was congruent with the observations that controlled cell death also had a highly conserved morphology, described as "apoptosis" [2]. This suggested that both the control of engagement of cell death and the execution of the death process itself were evolutionarily conserved.

SURVIVAL HIERARCHIES: SOME CELLS DIE MORE EASILY THAN OTHERS—A BRIEF REVIEW OF SOME OF THE PLAYERS DETERMINING SURVIVAL OR APOPTOSIS

The determinants of cell survival and of cell death are both extrinsic and intrinsic to the cell. Raff suggested [3] that all cells are in the default position of being able to undergo apoptosis but are prevented from doing so by signals from neighbouring cells and their environment within a multicellular organism. These signals arise through cell-to-cell contacts, from the extracellular matrix to which cells are attached [4–7] and from circulating survival factors, such as insulin-like growth factor I and nerve growth factor [8]. Removal of cells from their contextual neighbours, from their contextual extracellular matrix or from a milieu of survival factors (such as peptide growth/survival factors) results in apoptosis. Thus, the survival of any cell may be determined by the concentration of these factors, the number of receptors available for the ligand and by the topological position of the cell with respect to both its neighbours and extracellular matrix. Many of these survival components, and their "downstream" effectors, such as *BCR-ABL*, *RAS* and the IGF-1 receptor, are altered in malignancy. In a metastatic tumour cell, survival must be independent of the normal positional/topological context of a tissue. This implies that to become metastatic intrinsic mechanisms of survival (the avoidance of default position of cell death) must be initiated to allow survival away from normal "social" controls. Again, the implications of this for cytotoxic drug therapy are that tumour cells may be intrinsically more resistant to undergoing cell death than many normal cell types. In addition, there are external signals which engage apoptosis: ligation of the APO-1/fas receptor initiates a discrete cell death signalling cascade, presumably by removing the action of internal inhibitors of the default position of cell death [9]. The expression levels of both the death-promoting ligand and its receptor will again determine a hierarchy amongst different cells as to whether they may readily engage apoptosis.

A hierarchy of survival exists amongst cells according to the

relative provision of these various exogenous survival/death factors. The intrinsic determinants of a survival/death hierarchy are epitomised by members of the *BCL-2* family. The *BCL-2* gene was identified as a translocated product in follicular lymphoma. Transfection of *BCL-2* to myeloid cells did not provide a growth advantage nor allow growth in the absence of serum. Instead, expression of *BCL-2* suppressed the apoptosis stimulated by the withdrawal of serum survival factors [10, 11]. In the search for homologues of *BCL-2* a number of genes have now been discovered which encode both suppressors of apoptosis and accelerators of the process (reviewed in [12]). Bcl-2 is the archetypal member of a family of proteins which undergo homo- and heterodimerisations to each other via binding through conserved BH1, BH2 and BH3 domains [13]. The isolation of the Bcl-2 homologue Bax as a protein which immunoprecipitated with Bcl-2 [14], and the finding that its expression accelerated apoptosis suggested a model whereby Bax–Bax homodimers promote apoptosis whilst Bcl-2–Bax heterodimerisation inhibits apoptosis by limiting Bax–Bax homodimerisation [12, 13, 15]. How Bax–Bax homodimers accelerate apoptosis is unclear. Knowledge of the family of *BCL-2*-like genes has been expanding with recent discoveries of sequence-related promoters of apoptosis (bad, bak, bcl-X_s) and inhibitors of apoptosis (bcl-X_L) [14, 16–19]. The structural requirements for homo- and heterodimerisation and the degree of redundancy in binding were determined by the yeast two-hybrid system [20].

Taken overall, a model can be constructed whereby the survival of any particular cell type will be determined by the expression of a menu of genes which determine pro- or anti-apoptotic products. Although this menu is likely to be extensive, recent studies of ours into the survival hierarchies observed in the crypt epithelial cells of the small and large bowel have shown that the expression of *BCL-2* is a major determinant of survival in epithelial stem cells of the colon: the relative incidence of apoptosis in the crypts of the normal small intestine is much greater than that of the colon, either spontaneously or after DNA damage, and we observed bcl-2 protein only in the colonic epithelia [21]. The importance of the role of *BCL-2* expression was formally tested by observations of the amount of apoptosis observed in the epithelia of animals made homozygously null for the *BCL-2* gene: in these animals, spontaneous and DNA damage-induced apoptosis in the colon now resembled that of the small intestine, where normally *BCL-2* expression is absent [21].

Interestingly, cancers of the small intestine are extremely rare, suggesting a hypothesis that cancers develop preferentially in those cells which are capable of surviving DNA damage. These must have proliferative capability. Proliferation itself may be promoted by the carcinogenic process [22]. Observations such as these suggest that not only will the selectivity of DNA damage-induced cell death be determined by the menu of survival/death determinants in any cell type, but also that "survival thresholds" may be an important determinant of the genesis of cancer. This is one of the few ideas that ties fundamental events in carcinogenesis (the "survival" of DNA damaged cells) to resistance in chemotherapy (the "survival" of DNA damaged cells!).

INTRINSIC DRUG SENSITIVITY AND RESISTANCE AND ITS RELATIONSHIP TO CANCER INCIDENCE: A HYPOTHESIS AND MORE HIERARCHIES

Clinical experience suggests that there is a hierarchy of response to chemotherapy according to tumour type. Haematopoietic tumours are generally responsive and the carcinomas intrinsically resistant. Epidemiological data also show a hierarchy of tumour incidence, with the carcinomas being the major tumour types and those of haematopoietic tissue being few in number [22]. Superimposition of these observations provides an interesting idea (Figure 1). If it is important for malignant transformation that the cell has a relatively high survival potential, sufficient for it to survive DNA damage without deletion by apoptosis (cf. the relatively cancer-free small intestine which readily deletes damaged cells [21]), then perhaps the greater incidence of tumours arising from some epithelia is determined not only by the fact that they are exposed to an excessive carcinogenic risk, as surface-covering cells, but also that they have a high survival capability, determined by the expression of genes discussed above. One might expect that surface-covering cells exposed to the environment may naturally have a low threshold for the engagement of apoptosis. The prediction is that these common, high death threshold tumours would be resistant to chemotherapy—which they generally are—whereas those rarer tumours arising from haematopoietic cells might be more amenable to the engagement of cell death following cytotoxic therapy, which they are. The hypothesis places genes which determine survival and death "thresholds" at the very centre of the question of what might determine "intrinsic" drug sensitivity and resistance [23, 24].

THE INDUCTION OF APOPTOSIS BY ANTITUMOUR DRUGS: A CELLULAR "DECISION" NOT TO DIE AS A MAJOR FACTOR IN RESISTANCE

All of the anticancer drugs in the current pharmacopoeia can induce the morphological features of a programmed cell death, or apoptosis (reviewed in [23–27]). The original experiments both *in vivo* [28–29] and *in vitro* [30] had shown that the conserved nuclear change of chromatin condensation and margination at the nuclear periphery, and the formation of apoptotic fragments, which are engulfed by neighbouring cells, were associated with the death of both normal and tumour cells treated with many different types of cytotoxins or irradiation. It is therefore not surprising that the expression of genes which suppress apoptosis will suppress drug-induced apoptosis.

SUPPRESSION OF DRUG-INDUCED APOPTOSIS AND THE DANGERS OF THE "UNDEAD" CELL

There are two major classes of suppressors of apoptosis which have been shown to affect anticancer drug-induced

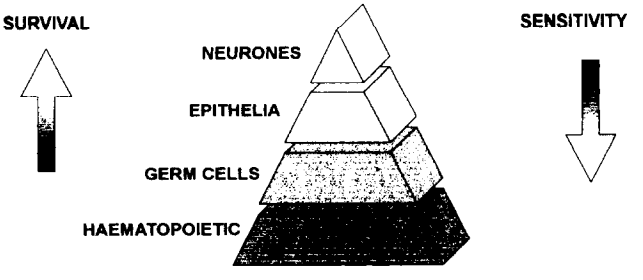

Figure 1. A suggested hierarchy of cellular survival in different tissues, based upon their relative sensitivities to DNA damaging agents. In this model, tumour incidence reflects the difficulty of cell deletion, and drug resistance the hierarchy of survival: epithelial tumours are therefore common and intrinsically more resistant to therapy, whereas haematopoietic tissue gives rise to few tumours but is intrinsically sensitive to cytotoxins, causing problems of host toxicity.

apoptosis, and so provide truly pleiotropic resistance: members of the *BCL-2* family of genes and components of survival signalling cascades.

The BCL-2 family of proteins

In a variety of cellular backgrounds, *BCL-2* (Table 1) and *BCL-X_L* expression has been shown to delay the onset of apoptosis induced by almost all classes of cytotoxic drugs. Most of the data from these experiments were accumulated by measuring changes in the short term viability of drug-treated cells which express *BCL-2* versus *BCL-2* non-expressers, either by vital dye exclusions assays or by temporal measurements of apoptosis, performed by scoring cells with condensed chromatin. What is singularly missing from many such experiments is clonogenic data to show that cells which do not engage an early drug-induced apoptosis are able to use their period of extended viability (being "undead") to repair damage-induced checkpoint controls and initiate proliferation, sufficient to provide significant numbers of viable daughter cells. Indeed, in a recent publication, the controlled expression of *BCL-2* in HeLa cells provided a delay in aphidicolin-induced apoptosis, but no clonogenic survival advantage [31]. However, in murine lymphoma cells, derived from a spontaneous tumour from *TP53* homozygous null animals, *BCL-2* expression produced an almost 10-fold resistance to 400 Rads of γ radiation in clonogenic assays. Delay in apoptosis was shown for a variety of other agents and it was claimed that the increase in clonogenicity, and not just a delay in cell death, extended to treatment with dexamethasone, etoposide, cisplatin and mitomycin-C [32]. Whether or not ectopic expression of *BCL-2* universally provides pleiotropic drug resistance, associated with the long-term survival of cells, is also complicated by findings that certain cell types do not appear to be protected by *BCL-2* apoptosis, but are instead protected by the expression of its homologue *BCL-X_L* [33–35]. It is possible that ectopic *BCL-2* cannot alone provide survival sufficient to promote clonogenicity when it is expressed against some genetic backgrounds; a number of accessory proteins are emerging as playing a role in the hetero- and homodimerisation of bcl-2 protein to itself and its pro-apoptotic partner bax, and it is possible that these are necessary for the provision of a sufficient delay in damage-induced apoptosis, allowing repair.

It could be claimed that the expression of *BCL-2* or *BCL-X_L* provides a genuine multidrug or pleotropic resistance, since its inhibition of drug-induced apoptosis crosses the entire spectrum of the pharmacopoeia, and not just the natural products. It is remarkable that cells treated with agents which induce considerable amounts of DNA damage maintain viability for considerable periods and may then, in some cases, go on to form colonies. The maintenance of viability with DNA damage in place, not only allows time for repair but, potentially, the fixation of mutations and chromosomal damage induced by the drugs. This is an extremely dangerous scenario, particularly if that tumour has a mutant p53 phenotype since this may promote genetic instability.

Are there strategies which can be used to counteract the suppression of drug-induced apoptosis by *BCL-2*? Ectopic expression of the death promoting Bcl-X_s protein in *BCL-2*-expressing MCF-7 human breast carcinoma cells sensitised them to the cytotoxicity of both etoposide and paclitaxel [36]. Strategies like this, delivering apoptotic accelerators such as bax and bcl-X_s or inhibitors of bcl-2, or bcl-X_L by expression of mimetics which prevent pro-apoptotic homologues from binding to bcl-2 protein, would seem to offer an important route for the circumvention of this type of pleotropic drug resistance.

Survival signals: inhibitors of drug-induced apoptosis

The survival of a cell in a multicellular organism is strongly dependant upon signals provided by other cells, as outlined in the Introduction. These arise from cell–cell contacts, cell–

Table 1. The effect of overexpression of BCL-2 *on the sensitivity of various cell types to the major classes of antitumour drugs*

Drug	Cell type	[Ref.]
Nitrogen mustard	Murine lymphoid FL5.12	[57]
Camptothecin, CPT-11	Murine lymphoid FL5.12; murine leukaemia L1210	[57, 58]
Etoposide	Murine bone marrow BAF3; human neuroblastoma NBL; murine S49.1 and WEHI17.2 T lymphoid; murine bone marrow; murine B cell lymphoma CH31	[48, 59, 60] [61, 62]
Cisplatin	Murine bone marrow BAF3; human neuroblastoma NBL	[59, 60]
Doxorubicin	Murine M1 leukaemia; murine bone marrow	[61, 63]
Gamma irradiation	p53–/– murine T lymphoma cells; murine bone marrow BAF3	[32, 59]
Fluorodeoxyuridine (and other thymidylate synthase inhibitors)	Human lymphoid MUTU-BL	[64]
2-Chloro-2′-deoxy adenosine	Human pre B cells (697)	[65]
Methotrexate	Murine M1 leukaemia; human pre B cells (697)	[48, 63]
Cytosine arabinoside	Murine M1 leukaemia; murine S49.1 and WEHI17.2 T lymphoid	[48, 63]
Dexamethasone	Human pre B cells (697); murine S49.1 and WEHI17.2 T lymphoid; human pre B cells (697)	[48, 66, 67]
Vincristine	Murine S49.1 and WEHI17.2 T lymphoid; human pre B cells (697)	[48, 67]
4-Hydroxy-cyclophosphamide	Human pre B cells (697)	[48]

matrix contact or by trophic factors. The prototypical survival factor is nerve growth factor which, in post mitotic cells, is necessary for the continued survival of neurones [37]. The delivery of discrete signals for survival, independent of those for proliferation, by so-called growth factors and by cytokines, was somewhat unexpected in a context wider than the neurotrophic factors [8]. Thus, the apoptosis-inducing ability of artificially elevating *C-MYC* expression in primary rat fibroblasts was quite specifically inhibited by insulin-like growth factor 1 (IGF1), which was a poor mitogen [38], and removal of cytokines such as IL-3 from a factor dependent haematopoietic cell line induced not only a cessation in proliferation but also apoptosis [39]. How these survival signals impinge on the engagement of apoptosis is unclear and their relative importance in the hierarchy of providing survival (whether they are "upstream" or "downstream" of *BCL-2* for example) is also unknown.

The effects of a discrete survival signalling pathway on drug sensitivity have been investigated using a temperature-sensitive mutant of the V-*abl* oncogene as a mimic of the *BCR-ABL* oncogene [40]. An IL-3 dependent murine mast cell line (IC2.9) underwent apoptosis when IL-3 was withdrawn at the non-permissive temperature for the expression of V-*abl* (39°) but maintained viability, without proliferation, in the absence of IL-3 but with v-*abl* active (32°). Treatment with melphalan at the non-permissive temperature provided complete protection against the onset of apoptosis over 120 h, at which time 100% of the cells not expressing active V-*abl* were dead. This complete maintenance of survival was more profound than any of the patterns of suppression of apoptosis observed in the presence of elevated levels of *BCL-2* (Table 1). The authors could find no change in the cellular levels of bcl-2 protein nor in its phosphorylated state. Analysis of the DNA damage showed that the "undead" cells had a full complement of DNA cross links [41]. Expression of V-*abl* elevated diacylglycerol levels and was associated with the specific translocation of protein kinase isoform βII to the nucleus, although how this might inhibit the engagement of apoptosis remains speculative [42]. The translocation of the *C-ABL* oncogene to form the *BCR-ABL* fusion (Philadelphia chromosome of chronic myelogenous leukaemia) also provides a significant survival advantage to drug treated cells and it is possible that the progression of CML is brought about by the survival of drug-treated cells.

The involvement of *RAS* in survival signalling was suggested by the earliest study of the effects of oncogenes on apoptosis [42], where it was suggested that levels of spontaneous apoptosis were suppressed by *C-H-RAS*. Expression of *C-H-RAS* in rat rhabdomyosarcoma cells provided a survival advantage after treatment with doxorubicin and, most importantly, gave a 3- to 5-fold increase in clonogenicity in soft agar [43]. The drug resistance of the *C-H-RAS* transfectants was not due to any change in the cell cycle of the cells nor to intracellular drug accumulation. Since mutational activation of *RAS* is a frequent event in human cancers, its involvement in the suppression of drug-induced cell death merits further attention.

"SENSING" DNA DAMAGE: THE ROLE OF TP53 IN DETERMINING DRUG SENSITIVITY AND RESISTANCE

The majority of antitumour drugs damage DNA, either directy or indirectly. The idea that this damage *per se* is not lethal but has to be "sensed" by the cell and coupled to the execution of apoptosis suggests that the failure of "sensors" could lead to drug resistance (as well as promoting carcinogenesis). The tumour suppressor *TP53*, the so-called "guardian of the genome" [44], has recently been suggested to be a direct "sensor" of DNA damage. It localises to single-stranded regions of DNA and to the termini of non-specific DNA templates [45]. p53 therefore acts as the "sensor" of DNA damage and the pattern of transcriptional regulation that ensues is critical in determining, according to cellular background, the outcome of a p53-dependent DNA damage response. One critical response is cell death but, according to cellular background, p53 can also initiate a cell cycle checkpoint which will promote repair of lesions which may be more lethal during DNA replication in S-phase [46]. As a transcriptional activator, p53 increases the transcription of *WAF-1*, producing an inhibitor of cell cycle progression at G1 [47]. Waf-1 is associated with the cyclin-dependent kinases required for G1 to S-phase progression so that functional p53 in a tumour (>50% of human tumours have been estimated to be mutant or null for *TP53*), might—paradoxically—promote survival, making p53-defective tumours more sensitive to DNA damage. However, this depends upon what other genes p53 is activating or suppressing. p53 has been shown to activate the transcription of the accelerator of cell death, bax (see above) [48]. Whether the promotion of transcriptional activity of p53 is important for the promotion of cell death was not supported by studies of Caelles and coworkers [48] who showed that DNA damage-induced, *TP53*-dependent apoptosis did not require new RNA or protein synthesis. Indeed, it was shown that Bcl-2 can relieve the p53 transcriptional repression and so inhibit cell death. Whatever the role of p53, loss of functional p53 may promote pleiotropic drug resistance to DNA damaging agents. The importance of p53 in promoting DNA damage-induced apoptosis was demonstrated by studies of immature thymocytes *in vitro* or intestinal epithelia *in vivo* from homozygous *TP53* null animals, generated by recombinant gene knockout procedures [49–51]. Cells which had been γ-irradiated did not undergo apoptosis in comparison with those which were homozygously *TP53* positive. *TP53* null thymocytes also failed to undergo apoptosis after treatment with the topoisomerase II inhibitor etoposide but, importantly, did undergo normal levels of apoptosis after treatment with the non-DNA damaging corticosteroid dexamethasone, suggesting that the non-DNA damage-induced pathway was discrete and p53 independent.

Lowe and colleagues [50, 52] showed that in fibroblasts, sensitised to undergo apoptosis by the expression of the adenovirus gene *E1A*, treatment with 5-fluorouracil, etoposide or doxorubicin was significantly less cytotoxic against a *TP53* null (–/–) background, 24–72 h after treatment. However, Kastan and colleagues [53] irradiated normal and *TP53* null murine fibroblasts (which were therefore isogenic) and determined that there was no difference in survival as measured by a clonogenic assay. However, the authors claimed that one possible difference between their result and those obtained using thymocytes [49, 50] was that fibroblasts did not undergo apoptosis, a somewhat curious suggestion. Irradiation of proliferating lymphoid cells from *TP53* null cells did not prevent apoptosis unless the cells were transfected with *BCL-2* suggesting that *TP53*-independent modes of cell death may be initiated in some cell types [32] and that *BCL-2* acts "downstream" of p53. Thus, strategies to restore *TP53* to

cells with a null phenotype or *TP53* mutations may not be effective if *BCL-2* is expressed. In a study of Burkitt's lymphoma and lymphoid cell lines, the mutation of *TP53* made cells significantly resistant to γ-irradiation, etoposide, cisplatin and nitrogen mustard as measured by viability (trypan blue exclusion) at 72 h after treatment. Whether this reflects a delay in the kinetics of cell death rather than true changes in sensitivity is unclear [54]. The potential of a *TP53* wild-type tumour to sustain DNA damage, but to enter the G1 checkpoint, and therefore to be resistant to DNA damaging agents, was subverted in experiments by O'Connor and colleagues by transfection of the papillomavirus type 16 *E6* gene, the product of which stimulates the proteolysis of the p53 protein, into human breast carcinoma cell line MCF-7 [54]. This cell line is *TP53* wild type but resistant to cisplatin. Expression of *E6*-induced sensitivity, not only because of the release from a G1 checkpoint but also because p53 upregulates Gadd45, a protein which stimulates excision repair. Downregulation of p53 therefore reduced excision repair as well as allowing cisplatin-damaged cells to enter S-phase. Cisplatin-induced DNA damage was insufficient to induce apoptosis in non-proliferating immature mouse thymocytes, a cell type that is exquisitely sensitive to irradiation and to etoposide [55]. However, if the small population of dividing thymocytes was examined, these were sensitive to cisplatin. This suggests that the quality of DNA damage is an important aspect of the signalling for the initiation of apoptosis. Presumably the intra-strand crosslinks induced by cisplatin, and the distortion they induced in DNA, are not "sensed" until replication forks collide with them, perhaps generating strand breaks. Similar conclusions were reached by Fan and colleagues [54] when studying the induction of a p53 G_1 checkpoint by γ-irradiation, etoposide, cis-platinum and nitrogen mustard: the former two agents induced a strong G_1 checkpoint compared with the latter two, presumably because p53 only detects strand breaks.

The significance of the mutation of *TP53* and of allelic loss to the progress of human neoplasia as an indicator of a poor prognosis is unquestionable. Whether the loss of p53 function alone is responsible for pleiotropic drug resistance to DNA-damaging drugs observed in many advanced cancers is doubtful. As multistage carcinogenicity progresses, it is likely that the survival potential of the cancer cell is increased by a variety of oncogenic events. Only as this occurred would the progressing tumour be able to survive the increasing genetic instability associated with malignancy. Even in the presence of wild-type *TP53*, drug sensitivity is not assured—it may operate to promote a checkpoint, permitting repair. The background in which p53 functions is critical. Restoration of p53 may indeed hold promise, but its position in the hierarchy (upstream of *BCL-2*) should not be forgotten (discussed with respect to chemotherapy in [32]). Nor should it be forgotten that *TP53* null cell lines such as the HL-60 myeloid leukaemia are exquisitively sensitive to DNA damaging agents, such as etoposide. There are as yet undiscovered p53-independent mechanisms of "sensing" DNA damage, and a hunt for these will yield further insights into resistance mechanisms where a DNA damage signal becomes uncoupled from an appropriate response. A likely candidate pathway involves the activation of the transcription factor, interferon regulatory factor (IRF-1), which is necessary for DNA damage-induced cell death in mitogen-activated T lymphocytes [56].

CONCLUSION

If it is the goal of chemotherapy to eliminate tumour cells with proliferative potential then a secure way of doing so is to kill them. Inducing cell death is not so simple, as the past forty years of drug research have told us, and as outlined in this paper. The idea that there are hierarchies of "thresholds" for cell death may explain the nature of "intrinsic" drug resistance. This idea fits neatly with ideas about the ease of tumour generation ("tough cells may be cancer prone"). At last the determinants of these hierarchies are being defined. How to modulate them selectively is a question familiar to many of us.

1. Vaux DL, Haecker G, Strasser A. An evolutionary perspective on apoptosis. *Cell* 1994, **76**, 777–779.
2. Wyllie AH. Cell death: a new classification separating apoptosis from necrosis. In Bowen ID, Lockshin RA, eds. *Cell Death in Biology and Pathology*. London, Chapman and Hall, 1981.
3. Raff MC. Social controls on cell survival and cell death. *Nature* 1993, **356**, 397–400.
4. Ruoslahti E, Reed JC. Anchorage dependence, integrins, and apoptosis. *Cell* 1994, 77, 477–478.
5. Frisch SM, Francis H. Disruption of epithelial cell-matrix interactions induces apoptosis. *J Cell Biol* 1994, **124**, 619–626.
6. Brooks PC, Montgomery AMP, Rosenfeld M, *et al.* Integrin $\alpha_4\beta_3$ antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. *Cell* 1994, **79**, 1157–1164.
7. Pullan S, Wilson J, Metcalfe A, *et al.* Requirement of basement membrane for suppression of programmed cell death in mammary epithelium. *J Cell Sci* 1996, **109**, 631–642.
8. Harrington EA, Fanidi A, Evan GI. Oncogenes and cell death. *Curr Opin Genet Dev* 1994, **4**, 120–129.
9. Nagata S. Apoptosis regulated by a death factor and its receptor: fas ligand and fas. *Phil Trans R Soc Lond (B)* 1994, **345**, 281–287.
10. Vaux DL, Cory S, Adams JM. *Bcl-2* gene promotes haemopoietic cell survival and cooperates with *c-myc* to immortalize pre-B cells. *Nature* 1988, **335**, 440–442.
11. Hockenbery D, Nuñez G, Milliman C, Schrieber RD, Korsmeyer SJ. Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. *Nature* 1990, **348**, 334–336.
12. Reed JC. Bcl-2 and the regulation of programmed cell death. *J Cell Biol* 1994, **124**, 1–6.
13. Yin X-M, Oltval ZN, Korsmeyer SJ. BH1 and BH2 domains of Bcl-2 are required for inhibition of apoptosis and heterodimerization with Bax. *Nature* 1994, **369**, 321–323.
14. Oltvai ZN, Milliman CL, Korsmeyer SJ. Bcl-2 heterodimerizes *in vivo* with a conserved homolog, bax, that accelerates programmed cell death. *Cell* 1993, **74**, 609–619.
15. Núñez G, Merino R, Grillot D, González-García M. Bcl-2 and Bcl-x: regulator switches for lymphoid death and survival. *Immunology* 1994, **12**, 582–588.
16. Yang E, Zha J, Jockel J, Boise LH, Thompson CB, Korsmeyer SJ. Bad, a heterodimeric partner for bcl-x_l and bcl-2, displaces bax and promotes cell death. *Cell* 1995, **80**, 285–291.
17. Kiefer MC, Brauer MJ, Powers VC, *et al.* Modulation of apoptosis by the widely distributed Bcl-2 homologue Bak. *Nature* 1995, **374**, 736–739.
18. Farrow SN, White JHM, Martinou I, *et al.* Cloning of a bcl-2 homologue by interaction with adenovirus E1B 19K. *Nature* 1995, **374**, 731–733.
19. Chittenden T, Harrington EA, O'Connor R, *et al.* Induction of apoptosis by the Bcl-2 homologue Bak. *Nature* 1995, **374**, 733–736.
20. Sato T, Hanada M, Bodrug S, *et al.* Interactions among members of the Bcl-2 protein family analyzed with a yeast two-hybrid system. *Proc Natl Acad Sci USA* 1994, **91**, 9238–9242.
21. Merritt AJ, Potten CS, Watson AJM, *et al.* Differential expression of bcl-2 in intestinal epithelia. *J Cell Sci* 1995, **108**, 2261–2271.
22. Clurman BE, Roberts JM. Cell cycle and cancer. *J Natl Cancer Inst* 1995, **87**, 1499–1501.
23. Dive C, Hickman JA. Drug-target interactions: only the first step in the commitment to a programmed cell death. *Br J Cancer* 1991, **64**, 192–196.

24. Fisher DE. Apoptosis in cancer therapy: crossing the threshold. *Cell* 1994, **78**, 539–542.
25. Hickman JA. Apoptosis induced by anticancer drugs. *Cancer Metast Rev* 1992, **11**, 121–139.
26. Dive C, Wyllie AH. Apoptosis and cancer chemotherapy. In Hickman JA, Tritton TR, eds. *Cancer Chemotherapy*. Oxford, Blackwell, 1993, 21–56.
27. Hickman JA, Potten CS, Merritt AJ, Fisher TC. Apoptosis and cancer chemotherapy. In Dexter TM, Raff MC, Wyllie AH, eds. *The Role of Apoptosis in Development, Tissue Homeostasis and Malignancy*. London, Chapman and Hall, 1994, 83–89.
28. Kerr JFR, Searle AJ, Harmon BV, Bishop CJ. In Potten CS, ed. *Perspectives on Mammalian Cell Death*. Oxford Science Publications, Oxford, 1987, 93–128.
29. Ijiri K, Potten CS. Response of intestinal cells of differing topographical and hierarchical status to ten cytotoxic drugs and five sources of radiation. *Br J Cancer* 1983, **47**, 175–185.
30. Searle J, Lawson TA, Abbott PJ, Harmon B, Kerr JFR. An electron-microscope study of the mode of cell death induced by cancer-chemotherapeutic agents in populations of proliferating normal and neoplastic cells. *J Pathol* 1975, **116**, 129–138.
31. Yin DX, Schimke RT. *BCL-2* expression delays drug-induced apoptosis but does not increase clonogenic survival after drug treatment in HeLa cells. *Cancer Res* 1995, **55**, 4922–4928.
32. Strasser A, Harris AW, Jacks T, Cory S. DNA damage can induce apoptosis in proliferating lymphoid cells via p53-independent mechanisms inhibitable by Bcl-2. *Cell* 1994, **79**, 329–339.
33. Datta R, Manome Y, Tanega N, *et al.* Overexpression of bcl-x_L by cytotoxic drug exposure confers resistance to ionising radiation-induced internucleosomal DNA fragmentation. *Cell Growth Diff* 1995, **6**, 363–370.
34. Chao DT, Linette GP, Boise LH, *et al.* Bcl-x_L and bcl-2 repress a common pathway of cell death. *J Exp Med* 1995, **182**, 821–828.
35. Gottschalk AR, Boise LH, Thompson CB, Quitáns J. Identification of immunosuppressant-induced apoptosis in a murine B-cell line and its prevention by bcl-x but not bcl-2. *Proc Natl Acad Sci USA* 1994, **91**, 7350–7354.
36. Sumantran VN, Ealovega MW, Nuñez G, Clarke MF, Wicha MS. Overexpression of Bcl-x_S sensitizes MCF-7 cells to chemotherapy-induced apoptosis. *Cancer Res* 1995, **55**, 2507–2510.
37. Levi-Montalcini R. The nerve growth factor: thirty-five years later. *EMBO J* 1987, **6**, 1145–1154.
38. Harrington EA, Bennett MR, Fanidi A, Evan GI. C-Myc-induced apoptosis in fibroblasts is inhibited by specific cytokines. *EMBO J* 1994, **13**, 3286–3295.
39. Williams GT, Smith CA, Spooncer E, Dexter TM, Taylor DR. Haemopoietic colony stimulating factors promote cell survival by suppressing apoptosis. *Nature* 1990, **343**, 76–82.
40. Chapman RS, Whetton AD, Dive C. The suppression of drug-induced apoptosis by activation of v-abl protein tyrosine kinase. *Cancer Res* 1994, **54**, 5131–5137.
41. Evans CA, Lord JM, Owen-Lynch PJ, Johnson C, Dive C, Whetton AD. Suppression of apoptosis by v-Abl protein tyrosine kinase is associated with nuclear translocation and activation of protein kinase C in an interleukin-3-dependent haematopoietic cell line. *J Cell Sci* 1995, **108**, 2591–2598.
42. Wyllie AH, Rose KA, Morris RG, Steel CM, Foster E, Spandidos DA. Rodent fibroblast tumours expressing human *myc* and *ras* genes: growth, metastasis and endogenous oncogene expression. *Br J Cancer* 1987, **56**, 251–259.
43. Nooter K, Boersma AWM, Oostrum RG, Burger H, Jochemsen AG, Stoter G. Constitutive expression of the c-H-*ras* oncogene inhibits doxorubicin-induced apoptosis and promotes cell survival in a rhabdomyosarcoma cell line. *Br J Cancer* 1995, **71**, 556–561.
44. Lane DP. p53, guardian of the genome. *Nature* 1993, **358**, 15–16.
45. Bayle JH, Elenbaas B, Levine AJ. The carboxyl-terminal domain of the p53 protein regulates sequence-specific DNA binding through its nonspecific nucleic acid-binding activity. *Proc Natl Acad Sci USA* 1995, **92**, 5729–5733.
46. Kuerbitz SJ, Plunkett BS, Walsh WV, Kastan MB. Wild-type p53 is a cell cycle checkpoint determinant following irradiation. *Proc Natl Aciad Sci USA* 1992, **89**, 7491–7495.
47. El-Diery WS, Tokino T, Veculesco VE, *et al.* WAF-1, a potential mediator of p53 tumor suppression. *Cell* 1993, **75**, 817–825.
48. Miyashita T, Reed JC. *bcl-2* gene transfer increases relative resistance of S49.1 and WEHI7.2 lymphoid cells to cell death and DNA fragmentation induced by glucocorticoids and multiple chemotherapeutic drugs. *Cancer Res* 1992, **52**, 5407–5411.
49. Clarke AR, Purdi CA, Harrison DJ, *et al.* Thymocyte apoptosis induced by p53-dependent and independent pathways. *Nature* 1993, **362**, 849–852.
50. Lowe SW, Schmitt EM, Smith SW, Osborne BA, Jacks T. p53 is required for radiation-induced apoptosis in mouse thymocytes. *Nature* 1993, **362**, 847–853.
51. Merritt AJ, Potten CS, Kemp CJ, *et al.* The role of p53 in spontaneous and radiation-induced apoptosis in gastrointestinal tract of normal and p53-dependent mice. *Cancer Res* 1994, **54**, 614–617.
52. Lowe SW, Bodis S, McClatchey LR, *et al.* p53 status and the efficacy of cancer therapy *in vivo*. *Science* 1994, **266**, 807–810.
53. Slickenmyer WJ, Nelson WG, Slebos RJ, Kastan MB. Loss of a p53-associated G1 checkpoint does not decrease cell survival following DNA damage. *Cancer Res* 1993, **53**, 4164–4168.
54. Fan S, Smith ML, Rivett DJ, *et al.* Disruption of p53 function sensitizes breast cancer MCF-7 cells to cisplatin and pentoxifylline. *Cancer Res* 1995, **55**, 1649–1654.
55. Evans DL, Tilby M, Dive C. Differential sensitivity to the induction of apoptosis by cisplatin in proliferating and quiescent immature rat thymocytes is independent of the levels of drug accumulation and DNA adduct formation. *Cancer Res* 1994, **54**, 1596–1603.
56. Tamura T, Ishihara M, Lamphier MS, *et al.* An IRF-1-dependent pathway of DNA damage-induced apoptosis in mitogen-activated T lymphocytes. *Nature* 1995, **376**, 596–599.
57. Walton MI, Whysong D, O'Connor PM, Hockenbery D, Korsmeyer SJ, Kohn KW. Constitutive expression of human bcl-2 modulates nitrogen mustard and campthothecin induced apoptosis. *Cancer Res* 1993, **53**, 1853–1861.
58. Kondo S, Yin D, Takeuchi J, Morimura T, Oda Y, Kikuchi H. *bcl-2* gene enables rescue from *in vitro* myelosuppression (bone marrow cell death) induced by chemotherapy. *Br J Cancer* 1994, **70**, 421–426.
59. Collins MKL, Marvel J, Malde P, Lopez-Rivas A. Interleukin 3 protects murine bone marrow cells from apoptosis induced by DNA damaging agents. *J Exp Med* 1992, **176**, 1043–1051.
60. Dole M, Nuñez G, Merchant AK, *et al.* Bcl-2 inhibits chemotherapy-induced apoptosis in neuroblastoma. *Cancer Res* 1994, **54**, 3253–3259.
61. Kondo S, Yin D, Morimura T, Takeuichi J. *bcl-2* gene prevents induction of apoptosis in L1210 murine leukemia cells by SN-38, a metabolite of the campthothecin derivative CPT-11. *Int J Oncol* 1994, **4**, 649–654.
62. Kamesaki S, Kamesaki H, Jorgensen TJ, Tanizaw A, Pommier Y, Cossman J. bcl-2 protein inhibits etoposide-induced apoptosis through its effects on events subsequent to topoisomerase II-induced DNA strand breaks and their repair. *Cancer Res* 1993, **53**, 4251–4256.
63. Lotem J, Sachs L. Regulation by bcl-2, c-myc, and p53 of susceptibility to induction of apoptosis by heat shock and cancer chemotherapy compounds in differentiation-competent and defective myeloid leukemic cells. *Cell Growth & Diff* 1993, **4**, 41–47.
64. Fisher TC, Milner AE, Gregory CD, *et al. Bcl-2* modulation of apoptosis induced by anticancer drugs: resistance to thymidylate stress is independent of classical resistance pathways. *Cancer Res* 1993, **53**, 3321–3326.
65. Gao X, Kundsen TB, Ibrahim MM, Haldar S. Bcl-2 relieves deoxyadenylate stress and suppresses apoptosis in pre-B leukemia cells. *Death and Differentiation* 1995, **2**, 69–78.
66. Alnemri ES, Fernandes TF, Haldar S, Croce CM, Litwack G. Involvement of bcl-2 in glucocorticoid-induced apoptosis of human pre-B-leukemias. *Cancer Res* 1992, **52**, 491–495.
67. Miyashita T, Reed JC. Bcl-2 oncoprotein blocks chemotherapy-induced apoptosis in a human leukemia cell line. *Blood* 1993, **81**, 151–157.