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Commentary

Modulating sensitivity to drug-induced apoptosis: the future for chemotherapy?

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Abstract

Drug resistance is a fundamental problem in the treatment of most common human cancers. Our understanding of the cellular mechanisms underlying death and survival has allowed the development of rational approaches to overcoming drug resistance. The mitogen activated protein kinase family of protein serine/threonine kinases has been implicated in this complex web of signalling, with some members acting to enhance death and other members to prevent it. A recent publication by MacKeigan *et al* is the first to demonstrate an enhancement of drug-induced cell death by simultaneous blockade of MEK-mediated survival signalling, and offers the potential for targeted adjuvant therapy as a means of overcoming drug resistance.

Keywords: apoptosis, drug resistance, MEK, paclitaxel

Improvements in chemotherapy for some malignancies, such as childhood leukaemia, have resulted in considerable increases in survival. However, many of the more common adult cancers, including carcinoma of the breast, remain stubbornly resistant to drug treatment, despite dose escalation and the increasing use of stem cell support [1]. The explosion of interest in apoptosis in the past 10 years has been underpinned by the hope that a greater understanding of the way in which cancer cells die after chemotherapy-induced damage would allow the development of a more rational approach to overcoming the problem of drug resistance. Unless cells receive external survival signals, they will 'default' to apoptosis [2]. These signals are provided by soluble cytokines and growth factors, cell–extracellular matrix contact and cell–cell contact. The overall survival threshold is probably determined by the balance of interactions between members of the Bcl-2 family of proteins on the cytoplasmic surface of internal membranes, such as the outer mitochondrial membrane. These pro-apoptotic or anti-apoptotic proteins can homodimerise or heterodimerise, and a satisfying but still unproven hypothesis is that, by doing so, they either activate or neutralise each other depending on the balance of death and survival stimuli.

A wide range of chemotherapeutic agents is able to trigger apoptosis (reviewed in [3]). In this model of their action, chemotherapeutic drugs drive cell death by generating damage signals at their locus of action (eg DNA damage), and these signals become integrated at Bcl-2 family protein containing complexes where the decision to undergo apoptosis is taken and signalled to apoptosis effector molecules. This is classical stimulus response coupling, and it is the efficiency of this coupling that determines the threshold for

ERK = extracellular signal-related kinase; IL = interleukin; JNK = c-Jun N-terminal kinase; MAP = mitogen activated protein; MEK = MAP kinase kinase; MEKK = MEK kinase; MKK = MAP kinase kinase.

survival. A complex of proteins, describing this coupling and activation centre, has been conceptualised as an 'apoptosome', and contains the precursors of the proteolytic enzymes (caspases) that cleave key cellular proteins to generate the apoptotic morphology [4]. While many mechanisms of drug resistance have been defined in which drug–target interactions are modified, it is also important to consider the impact of the cellular environment on the coupling of drug-induced damage to the activation of the 'apoptosome', via modification of Bcl-2 family proteins.

Cell survival *in vivo* depends on ligation of surface receptors by soluble factors and both cell–cell and cell–matrix interactions. The signalling pathways activated by these receptors ultimately impinge upon Bcl-2 family members, and this may be a key mechanism mediating drug resistance. Such a survival signalling pathway exists between interleukin (IL-3) receptor ligation and the pro-apoptotic protein Bad [5]. It is thus possible that signals from growth factors and cytokines provide not only mitogenic cues, but also discrete survival signals that raise the survival threshold of tumours and contribute to drug resistance. Loss of IL-3 in pro-B lymphocytes leads to the upregulation of the pro-apoptotic Bcl-2 family protein Bim, via the Forkhead transcription factor FKHL-1, and this induces apoptosis [6]. In a B-cell lymphoma model, the provision of extrinsic survival signals attenuates etoposide-induced exposure of the N-terminus of Bax, an early step in the activation of this pro-apoptotic protein [7]. In the mouse mammary epithelial cell model, loss of cell–substrate contact-mediated ligation of integrin receptors results in a conformational change in the N-terminus of Bax, and its subsequent translocation to the mitochondria [8]. These conformational changes in Bax, and its subsequent translocation to mitochondria, are mediated by p38 mitogen activated protein (MAP) kinase in nitric oxide induced apoptosis in neurons [9]. A further link between protein serine/threonine kinase signalling and this part of the cell death response is provided by the recent observation that c-Jun N-terminal kinase (JNK) is involved in the coupling of DNA damage to mitochondrial cytochrome *c* release in fibroblasts [10].

This brings us neatly from the general concepts of apoptosis and cell survival to the specific roles of the extracellular signal-related kinase (ERK)/MAP kinase family of protein kinases in the regulation of cell death. This family of kinases consists of proline-directed serine/threonine kinases that are activated by dual phosphorylation on tyrosine and threonine, and that are widespread among living organisms. In mammals, they exist mainly in cascades containing three kinases functioning in series. In broad terms, there are three distinct families. In the ERK1/ERK2 module, growth factor derived extracellular signals are translated to Raf-1 activation, which leads to the phosphorylation of MAP kinase kinase (MEK)1 and MEK2; these in turn phosphorylate and activate ERK1 and ERK2. In the stress-activated protein kinase/JNK module, a wide range of stimuli including UV light and osmotic shock result in activation of MEK kinase (MEKK)1, and then MAP kinase kinase (MKK)4 and MKK7, which phosphorylate JNK. The final member of the family is p38 MAP kinase, which is also activated by stress and inflammatory cytokines such as tumour necrosis factor-α and IL-1, MEKK1 and MKK3 and MKK4 (for a review, see [11]). Although these pathways share many similarities, they are clearly independent: MEK1/MEK2 do not phosphorylate JNK or p38, and MKK3/MKK4/MKK7 do not phosphorylate ERK1/ERK2 [12]. A widely accepted model is that the balance between growth factor activated ERK and stress-activated JNK and p38 pathways determines whether the cell lives or dies. In the rat phaeochromcytoma cell line PC-12, withdrawal of nerve growth factor leads to sustained JNK and p38 MAP kinase activity, inhibition of ERK activity, and apoptosis, which can be prevented by transfection of constitutively active MEK1 mutants [13]. Hippocampal neurons in *jnk 3* knockout mice do not undergo excitoxin-induced apoptosis [14], and JNK is needed for apoptosis of immature T lymphocytes in developing mice [15]. JNK is also needed for UV-induced apoptosis in mouse fibroblasts, which intriguingly is mediated via a failure of mitochondrial cytochrome *c* release, suggesting further interaction between the Bcl-2 family and the MAP kinase signalling cascades [10]. Further subtlety is lent to this system by the discovery that, while MEKK1$^{-/-}$ embryonic stem cell lines lose their JNK response to microtubule disruption and cold shock, this kinase is not needed for JNK activation by UV irradiation or heat shock [16]. Furthermore, this loss of MEKK1-mediated activation of JNK leads to an increased apoptotic response to hyperosmolarity and microtubule disruption, suggesting that the survival or death specificity of these pathways may depend upon the precise nature of the signal inducing them. JNK does not appear to be needed for Fas-mediated or tumour necrosis factor-mediated apoptosis [17] and, in some situations, may protect cells from apoptosis.

The discovery of small molecule inhibitors of the MAP kinase pathways facilitated the transition from the theoretical framework already described to the implementation of novel therapies. The first inhibitor to be identified was PD 098059, which inhibits MEK without affecting JNK or p38, and is able to prevent call growth and reverse the phenotype of *ras*-transformed cells [18]. U0126 is a further non-competitive MEK1/MEK2 inhibitor, which is also specific for these kinases. The major advantage of U0126 over PD 098059 is its 100-fold greater affinity for MEK, thus making it more suited to *in vivo* applications [19]. The efficacy of this approach has been demonstrated *in vivo* with an even more effective MEK inhibitor, PD 184352. This compound is able to completely inhibit MEK activity in a range of human tumour cell lines at concentrations as low as 100 nM, without effecting JNK or

p38 activity. This compound was active *in vivo* against xenografts from a range of tumour cell lines, its activity correlating with the expression levels of MEK within the cell lines [20]. This overexpression of MEK within experimentally induced tumours has also been demonstrated *in vivo* in certain types of brain tumours and, pertinently, in breast carcinoma [21]. Thus, not only is the theoretical basis for the use of MEK inhibition to modulate survival signalling in place but effective drugs are available, and the target is overexpressed in breast cancer. But with which chemotherapeutic drugs should these inhibitors be used?

The alkaloid drug paclitaxel (Taxol) is a relatively novel anticancer agent. Paclitaxel has a wide range of activities *in vivo* against relatively drug-resistant solid tumours and a unique mode of action. Unlike the *Vinca* alkaloids, paclitaxel binds preferentially to polymerised tubulin and shifts the dynamic balance between tubulin dimers and microtubules towards microtubule assembly. Paclitaxel ultimately causes mitotic block at the metaphase/anaphase boundary by the suppression of dynamic instability (the transition between phases of lengthening and shortening) at the ends of mitotic spindle microtubules [22]. Paclitaxel treatment of lymphoblasts in culture results in a rapid increase in JNK activity and a reduction in ERK2 activity [23]. Bcl-2 can also be phosphorylated by JNK [24], providing a further link between paclitaxel, MAP kinase cascades and Bcl-2 family proteins. The *in vivo* significance of these observations remains to be clarified.

MacKeigan *et al* [25] have used the theoretical framework discussed in this paper to augment the pro-apoptotic activity of paclitaxel in breast, lung and ovarian carcinoma cell lines. They show that treatment with nanomolar concentrations of paclitaxel results in increases in JNK and ERK1/ERK2 activity, and that the latter is specifically blocked by micromolar concentrations of the MEK inhibitor U0126. Paclitaxel-induced apoptosis is significantly increased by U0126, PD98059, or by dominant-negative MEK, and this effect is considerably more than additive. This logical approach to the modulation of drug-induced apoptosis is exactly what was hoped would arise from an understanding of cell death, illustrating well the principle that apoptosis results from either loss of survival signals (provided in this setting by ERKs) or death signals (in the present study from JNK). The power of the approach of MacKeigan *et al* [25] is partly that it tilts both sides of the balance, both activating death signals and inhibiting survival signals, but that they are also able to demonstrate that clinically relevant concentrations of drug can be made dramatically more potent with readily available agents. With the *in vivo* efficacy of small molecule MAP kinase inhibitors already demonstrated, their use to enhance the efficacy of chemotherapeutic agents in clinical trials is a realistic goal. Perhaps the future of chemotherapy is no longer so bleak.

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