Survival signals within the tumour microenvironment suppress drug-induced apoptosis: lessons learned from B lymphomas

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Abstract

The suppression of apoptosis is one mechanism by which tumours become drug resistant. Extracellular signals from the germinal centre (GC) of secondary lymphoid tissue can rescue B cells from physiological- and chemotherapy-induced apoptosis. Such survival signals include CD40 receptor ligation, interleukin-4 (IL-4) receptor stimulation and the interaction of the integrin ligand VCAM-1 with its receptor. The GC environment was modelled in vitro by providing B lymphoma cells with these survival signals. JLP119 B lymphoma cells underwent apoptosis after exposure to the topoisomerase II inhibitor etoposide and this was dramatically reduced when the cells were cultured in the GC system. CD40 receptor ligation resulted in increased levels of Bcl-XL. Etoposide diminished the binding between Bax and Bcl-XL but this was restored by IL-4 and VCAM-1 triggered signals. These data demonstrate combined effects of three microenvironmental signals on the Bcl-2 family and illustrate the potential importance of such signalling pathways in drug resistance of tumour cells.

Introduction

Cells require survival signals to prevent the engagement of apoptosis (Raff 1992). In vivo, cell survival is mediated by signalling via ligation of surface receptors by soluble factors and by cell-cell and cell-matrix interactions within a specific microenvironment. Because members of the Bcl-2 family of proto-oncogenes play a central role in the regulation of apoptosis (Reed 1997, Kroemer 1997), it seems most likely that these may be the ultimate recipients of signalling pathways initiated by microenvironmental survival stimuli. An established precedent lending support to this idea is the inactivating phosphorylation of the pro-apoptotic Bcl-2 family member, Bad, by protein kinase B (Akt) downstream of interleukin (IL)-3 receptor ligation (Del Peso et al. 1997). We have examined the role of microenvironmental signals on members of the Bcl-2 family and drug sensitivity of human B lymphoma cells (Walker et al. 1997). Suppression of drug-induced apoptosis, despite imposition of drug-induced damage, is considered to be a mechanism for pleotropic drug resistance of tumour cells (Dive & Hickman 1991, Fisher 1994). However, apoptosis was found only to be delayed in many studies of the effects of (non-physiological) hyperexpression of anti-apoptotic members of the Bcl-2 family on tumour cell fate after drug treatment in vitro (Dive & Hickman 1991, Yin & Schimke 1995). We suggest that endogenous Bcl-2-like molecules and their respective binding proteins might function fully to suppress drug-induced apoptosis only when cells in which these proteins are expressed are stimulated by an appropriate combination of survival signals such as those that would be presented to that cell in vivo. Moreover, if anti-tumour strategies based upon removal of a blockage in the coupling of drug-induced damage to the engagement of tumour cell apoptosis are to succeed, the contribution of combined and integrated micro-environmental signals to drug resistance must be better understood. This should permit identification of nodal points on survival signalling pathways that could represent useful anti-tumour drug targets.

The B lymphoma cell model in vivo

In vivo, B lymphoma cells in secondary lymphoid tissues would be exposed to germinal centre (GC)-derived signals
Survival signalling pathways initiated in this way include those generated by activation of the B cell surface molecule, CD40, by ligation of the IL-4 receptor (IL-4R) and by stimulation of VLA4, the $\alpha_4\beta_1$ integrin receptor. Low-grade follicular B lymphomas initially regress dramatically after combination chemotherapy, but patients inevitably relapse, with a drug-resistant tumour located at the same site as the original disease (Horning 1994). A critically important question remains unanswered: when the tumour regresses after cytotoxic chemotherapy, why does clinically undetectable minimal residual disease remain? During the cellular carnage brought about by a combination of cytotoxic drugs within and around the tumour site, survival signals derived from lymphoma cell contact with other cells and with stroma may now be only very heterogeneously distributed within the GC. Lymphoma cells, which remain alive, may do so, at least in part, because they are located within what we previously termed a survival niche (Dive & Hickman 1991), where they still receive enough microenvironmental survival signals to resist drug-induced apoptosis.

The B lymphoma cell model in vitro using an in vitro system developed in our laboratory (Fig. 1), we demonstrated that the provision of GC-derived cell survival signals (to activate CD40, VLA4 and IL-4R) increased the clonogenicity of human B lymphoma cells that had been exposed to DNA damaging drugs. In contrast, the enforced hyperexpression of Bcl-2 in the absence of these signals did not provide any clonogenic survival advantage after drug-induced DNA damage (Dive & Hickman 1991). One hour of exposure to etoposide (40 µM) induced apoptosis (80% at 72 h) that was reduced to 20% when cells interacted with immobilised anti-CD40 antibody, IL-4 and VCAM-1 (the ligand of VLA4) (Taylor et al. 1998). These signals did not prevent etoposide-induced p53 stabilisation and p21WAF1, associated cell cycle arrest. Each survival signal delivered alone suppressed etoposide-induced apoptosis, but the combination of all three GC-derived signals yielded more surviving lymphoma cells. CD40 signalling increased the mRNA and protein levels of the anti-apoptotic Bcl-2 family member, Bcl-XL. This was mediated via the transcription factor, NF-$\kappa$B, and inhibition of the nuclear translocation of this transcription factor partially restored sensitivity to etoposide-induced apoptosis. IL-4 had no effect on Bcl-XL levels, but accelerated the upregulation of this survival protein by the CD40 signal. VCAM-1- and IL-4-mediated signals diminished a drug-induced change in the conformation of the pro-apoptotic protein, Bax. This conformational change was analysed in intact cells by flow cytometry using an N-terminal epitope-specific anti-Bax monoclonal antibody that is undetectable in untreated cells but unmasked after DNA damage before the onset of
morphological apoptosis. This drug-induced change in Bax conformation correlates with its disassociation from Bcl-XL (authors’ unpublished observations). Taken together, the data suggest that the three GC-derived signals work in concert to suppress drug-induced apoptosis according to the following model: IL-4- and VCAM-1-generated signals promote Bcl-XL-Bax binding and CD40 signals to increase the amount of Bcl-XL available to bind Bax. The implication from this is that drug-induced damage results in an increase in unrepressed Bax that is lethal.

These data demonstrate integrated effects of three microenvironmental signals on the Bcl-2 family and drug resistance of B lymphoma cells. The study emphasises the need to include microenvironmental factors when considering the multiple mechanisms that govern tumour cell responses to chemotherapy. In this project, we attempted to model, in vitro, a disease (low-grade B lymphoma) in which a drug-resistant tumour re-emerges within the same microenvironment as the original neoplasm. In low-grade follicular lymphoma, the process of tumour relapse takes several years. One can only presume that the clinically undetectable subpopulation of cells that did not die by apoptosis after chemotherapy lie dormant and, at some stage when damage is repaired sufficiently, these cells re-enter the cell cycle and slowly divide to repopulate a drug-resistant tumour. Throughout this process, these tumour cells would continue to receive GC-derived survival signals. When considering breast tumours, it will be important to know the identity, not only of the local factors that signal for cell survival in the breast, but also of microenvironmental cues within those distant sites where metastases lodge, survive and grow.

References
Del Peso L, Gonzalez-Garcia M, Page C, Herrera R & Nunez G 1997 Interleukin-3 induced phosphorylation of Bad through protein kinase Akt. Science 278 687-689.