Drug resistance remains a serious limiting factor in the treatment of acute myeloid leukemia (AML) either at initial presentation or following primary or subsequent relapses. Using specific kinase inhibitors, this study has investigated the contribution of the Raf/MAP kinase regulated survival pathways to drug resistance, and resulting suppression of apoptosis. Inhibition of the Raf/MAP kinase pathway with Apigemin did not sensitize pro-myelocytic HL-60 cells to drug-induced apoptosis, suggesting a lack of involvement for this pathway in drug resistance. In contrast, the use of two specific PI3-kinase inhibitors, LY294002 and Wortmarnun, did cause a significant increase in apoptosis in combination with cytotoxic drugs. Following this observation, the contribution of two downstream effectors of PI3-kinase, p70S6-kinase and PKB/Akt, were examined at different stages of the cell. The hsp70 expressing cells, treated with these inhibitors, were found to be more resistant to the downstream PI3-kinase regulators Bad.

Hsp70 increases resistance to morphological apoptosis by inhibiting p70S6-kinase with rapamycin did not alter levels of drug-induced apoptosis. In contrast PI3-kinase inhibition led to significant dephosphorylation of downstream kinase PKB, suggesting that the PI3-kinase/PKB survival pathway may play a major role in chemoresistance in AML. This pathway has previously been associated with modifications of the apoptotic regulator Bad. However we found no evidence of Bad heterodimer formation with anti-apoptotic regulators Bcl-2, Bcl-X, or Mcl-1, nor of alterations in Bax/Bcl-2 or Bax/Mcl-1 heterodimers following PI3-kinase inhibition. This suggests that alternative targets of PI3-kinase/PKB, distinct from the Bcl-2 family may be responsible for contributing to drug resistance in AML. This work may represent a novel strategy for treatment of multi-drug resistance in myeloid leukemia by combined use of specific protein kinase inhibitors with conventional chemotherapeutic drugs.

**12** PI3-kinase survival signals contribute to multi-drug resistance in myeloid leukemia independently of Bad heterodimer formation.

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**13** Stress proteins and the regulation of apoptosis in tumour cells.

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Tumour cells often express elevated levels of heat shock protein 70 (hsp70). Increased expression of hsp70 is associated with progression of many human cancers. The selective protection of hsp70 against oxidative stress inducing agents has been previously described, however, the specific molecular mechanism underlying hsp70’s protective effect remains undefined.

In the present study, hsp70 transfected Jurkat T cells were treated with the cytotoxic agents hydrogen peroxide and actinomycin D, both of which have been shown to cause a reactive oxygen burst within the cell. Hsp70 increases resistance to morphological apoptosis induced by both cytotoxic agents. Immunofluorescent studies revealed that resistance to apoptosis is occurring when the hsp70 protein is localised to the cytosol of the cell. The hsp70 expressing cells, treated with these cytotoxic agents, were examined at different stages along the apoptotic pathway, in an attempt to position where in the apoptotic cascade the hsp70 protein is mediating its effect.

We have established that hsp70 mediated protection is occurring upstream of caspase activation and mitochondrial depolarisation, suggesting that hsp70 is mediating its protective effect at an early stage of the apoptotic process.

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**14** Prion Peptide Stimulates Apoptosis In Human Neuronal Cells

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The prion diseases are a group of transmissible degenerative diseases of the central nervous system which can be characterized by accumulation in the brain of the scrapie isoform (PrPSc) of the cellular prion protein (PrPc). A fragment of the prion protein (PrP106-126), which retains many of the characteristics of PrPSc, and is central to all identified disease causing isoforms of the prion protein, has previously been shown to induce apoptosis in rat neuronal cells in culture. Apoptosis is a cellular suicide pathway which has been implicated in a number of neuropathological conditions. In order to study the mechanism of apoptosis induced by PrP106-126 on a human system, we used the human neuroblastoma cell line SH-SYSY. PrP106-126 was found to induce cell death in a dose dependent manner, and this death was determined to be occurring via apoptosis. PrP106-126 was found to rapidly induce a decrease in mitochondrial membrane potential, concomitant to a disappearance of Bcl-2. Furthermore, this downregulation of Bcl-2 was found to be inhibited by the caspase inhibitor z-VAD. Mitochondrial depolarisation was not inhibited by z-VAD. Effects on the levels of other Bcl-2 family members were also examined. The effects of the peptide were specific as no changes were observed upon treatment with a scrambled version of the peptide (PrP106-126 csr.). This study sheds some light on the intracellular mechanisms by which neurons may undergo cell death during prion disease. Understanding how prions induce neuronal death may lead to important therapeutic strategies in the future.

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**15** INVESTIGATION OF THE EFFECTS OF HUMAN SERA FROM NEWLY DIAGNOSED DIABETIC PATIENTS ON CELL DEATH

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Destruction of the pancreatic beta cell in vivo is the result of a sustained attack by cellular and soluble factors of the immune system. Apoptosis may be at least partly responsible for this beta cell death in vivo. However, the type of beta cell death provoked by serum soluble factors in vivo is still open to speculation. Aims: To determine whether serum from newly diagnosed Type 1 diabetic subjects caused DNA damage or cell death by apoptosis in vitro. Methods: Human Jurkat T cells and human islet single cells were cultured with sera from at least seven control and seven diabetic patients. Cell membrane integrity, DNA strand breakage and chromatin condensation were recorded using cytochemical staining and single cell gel electrophoresis. Results: The percentage of Jurkat cells which were intact and non-apoptotic was slightly but significantly reduced by diabetic sera from 93.3±0.6% to 90.3±0.9% (P<0.05; N=11). Corresponding values in cytokine or etoposide treated Jurkat cells were 83.7% and 75.2% respectively. Apoptosis and necrosis levels were 1.0±0.2 and 2.1±0.4 after culture in control sera, 1.9±0.4 and 3.1±0.9 in DM sera; 8.5±2.4 and 3.9±1.8 in cytokine-treated Jurkat cells in the same experiments. However, DM sera (10%, 48h) increased cell death by necrosis in human islet single cells from 14 to 39% (cytokine to 16%) without significant apoptosis (P=0.05; N=4). Conclusion: Sera from diabetic patients caused a significant increase in cell death in vitro in Jurkat T cells and in human islet cells.

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