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Apoptotic response and the mechanism of resistance to camptothecin: a study of gene expression and functional effects

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RC0.1, a camptothecin-resistant derivative of the DU145 prostate cancer cell line, known to contain an R364H point mutation in topoisomerase I (top1). To assess gene expression changes that might also be involved in the acquisition of RC0.1,s drug resistance, we used "Oncochip" cDNA microarrays to compare the two cell lines with respect to expression of 1,650 cancer-related genes. Differences in transcript expression level > 1.5-fold were found for 181 genes. This level of difference was demonstrated to be statistically reliable using the stratum-adjusted Kruskal-Wallis test, after taking into account a dye-dependent variable. These differences in expression were concentrated in a statistically significant way ($p<0.05$) in 5 out of 182 functional categories. The largest of these categories was that of "apoptosis" genes. To assess whether or not RC0.1 had developed generalized resistance to apoptosis, we assessed the cells' responses to multiple, disparate apoptotic triggers, including camptothecin, staurosporine, serum starvation, cisplatin, UV irradiation, and gamma irradiation. Flow cytometric Annexin-V and TUNEL assays provided indices of early and late stages of apoptosis, respectively. For all stimuli, reduced levels of both phosphatidylserine translocation (by Annexin-V) and DNA strand breakage (by TUNEL) were observed for RC0.1, providing functional evidence for generalized resistance to apoptosis. When the genes with expression levels altered in RC0.1 were located on maps of molecular interactions in the cell, it became evident that there were two coherent blocks of genes concentrated in the core apoptotic and Akt-related subsystems. The survival molecules PI3K, Akt and Bcl-2 were upregulated in RC0.1, and the of the apoptotic BAD and caspase 6 molecules down-regulated. As a first test of the functional significance of these observations, we exposed the cells to wortmannin, a natural product that blocks the activity of PI3K. Consistent with the proposed involvement of this portion of the pathway in resistance to the apoptotic triggers employed, wortmannin treatment increased the apoptotic response of DU145, but not RC0.1, following treatment with camptothecin. These findings demonstrate major differences between DU145 and RC0.1 in apoptotic machinery, independent of the mutation in topoisomerase 1.

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Role of MAPKs in heat-induced apoptosis

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External insults have been demonstrated to activate a cytoplasmic signaling system mitogen-activated protein kinase (MAPK) pathway, i.e. ERK1/2, JNK and p38 kinase. Although heat-shock has been demonstrated to stimulate MAPKs, the exact role of MAPKs in heat-induced cell death is unknown. In the present study, we have investigated possible relationship between the activation of MAPKs and apoptosis caused by heat-shock in HL60 human promyelocytic leukemia cells. The activation of MAPKs was examined with immunocomplex kinase assay and western blot analysis and the apoptosis was estimated from the DNA fragmentation using agarose gel electrophoresis and also from the cellular DNA content using flow cytometry. Heating HL-60 cells at 43°C for 1 h caused significant apoptosis and varying degrees of activations of the MAPKs. The most pronounced activation occurred in JNK, which was activated as much as 15 times at 1 h after heating. The heat-induced JNK activation could be suppressed by pre-treating the cells with JNK inhibitor II. Importantly, the JNK inhibitor also significantly suppressed the heat-induced apoptosis. The heat-induced activation of p38 was far less than that of JNK, and pre-treating the cells with p38 inhibitor SB203580 did not significantly suppress the heat-induced apoptosis. ERK1/2 were slightly activated by heat-shock and PD 98059, an inhibitor of ERK1/2, had little effect on the heat-induced apoptosis. It has been known that an acidic environment can induce apoptosis by itself and enhances heat-induced apoptosis. In the present study, the acidic stress alone did not significantly activate MAPKs, but it increased the heat-induced activation of MAPKs. It is concluded that among the MAPKs, JNKplays the major role in heat-induced apoptosis, and than an acidic environment increases the heat-induced apoptosis probably by increasing the activation of JNK.

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Aplidin induces the mitochondrial apoptotic pathway via oxidative stress-mediated JNK and p38 activation and protein kinase C delta

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Aplidin™, a new antitumoral drug presently in phase II clinical trials, has shown both *in vitro* and *in vivo* activity against human cancer cells. Aplidin™ effectively inhibits cell viability by triggering a canonical apoptotic program resulting in alterations in cell morphology, caspase cascade activation, and chromatin fragmentation. Pro-apoptotic concentrations of Aplidin™ induce early oxidative stress, which results in a rapid and persistent activation of both JNK and p38 MAPK and a biphasic activation of ERK. Inhibition of JNK and p38 blocks the apoptotic program induced by Aplidin™, demonstrating its central role in the integration of the cellular stress induced by the drug. JNK and p38 MAPK activation results in downstream cytochrome-c release and activation of caspases -9 and -3 and PARP cleavage, demonstrating the mediation of the mitochondrial apoptotic pathway in this process. We also demonstrate that protein kinase C delta (PKC-d) mediates the cytotoxic effect of Aplidin™ and that PKC-d is concomitantly processed and activated late in the apoptotic process by a caspase-3 mediated mechanism. PKC-d appears as a key component necessary for full caspase cascade activation and execution of apoptosis, which most probably initiates a positive feedback loop further amplifying the apoptotic process.

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The impact of c-Src on drug sensitivity/resistance in human colon cancer cells

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c-Src expression level and/or activity is elevated early in the progression of colon cancer, a tumour that is commonly inherently resistant to chemotherapy. The participation of c-Src in signaling pathways may be important for the development of this malignancy, including those that promote proliferation, invasion and metastasis. v-Src both primes for and suppresses apoptosis in fibroblasts (Johnson et al 2000, Webb et al 2000). Here we have addressed the involvement of c-Src in determining drug sensitivity/resistance in the human colon carcinoma cell line KM12C transfected with constitutively activated c-Src (Y527F) to generate sublines 2C3 and 2C4 which had 4 and 10 fold higher levels of Src expression respectively than the empty vector control (2CV). 2C3 and 2C4 also expressed elevated levels of the anti-apoptotic Bcl-2 protein, though no other changes in the expression levels of Bcl-xL, Bax, Bak or Bad were observed. Elevated Bcl-2 expression suggested that 2C3 and 2C4 cells might display enhanced resistance to drug-induced apoptosis. However, increased sensitivity to a panel of anticancer drugs was observed as illustrated by the EC50 values below. The mode of oxaliplatin-induced KM12 cell death was confirmed as apoptosis based on changes in nuclear morphology. Sensitisation to oxaliplatin and cisplatin-induced apoptosis was also observed in KML4A cells (metastatic variant of KM12C) transfected with kinase-dead c-Src (MF-10) or the SH3-SH2 domains of c-Src (251-6, 251-13) as compared to the vector control (pBABE-1).

Src Construct	EC_{50} (μM)		
	Oxaliplatin	Cisplatin	Etoposide
Active – 2C3/2C4	65	75	4.8
Vector – 2CV	175	125	7.5
Kinase Dead – MF-10	75	70	–
SH3-SH2 – 251-6, 251-13	80	70	–
Vector – pBABE-1	175	95	–