JNK substrate. Using jnk1-deficient MEFs and the inhibitor SP600125, we also show that specifically JNK1 is required for cisplatin-induced apoptosis. However, although Bak is required for apoptosis, JNK1 was not required for the conformational activation of Bak. Furthermore, a dominant-positive mutant of MEKK1 could activate Bak also in the absence of JNK1. We demonstrate that the role of JNK1 is instead to provide the signal for already activated Bak to form the high-molecular weight complexes likely involved in cytochrome c release. Thus, in the absence of JNK1 activity, Bak activation is not necessarily a marker for apoptosis. Finally, we show that apoptosis correlates with Bak complexes in the range of 80–170 kDa, but not with larger complexes which are formed also in the absence of JNK activity and apoptosis.

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Variable distribution of TRAIL Receptor 1 in primary human tumor and normal tissues

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Background: TRAIL-Receptor 1 (TRAIL-R1/Death-receptor 4) has been reported to be selectively and generally expressed in tumors. The objective of this study was to identify specific malignancies that may be most likely to express this receptor. Such malignancies could be strong candidate indications for TRAIL-R1 specific therapeutics in development, such as HGS-ETR1, a fully human agonistic TRAIL-R1 mAb. The current study utilizes a highly specific immunohistochemical assay to evaluate TRAIL-R1 in human tumor and normal tissues.
Methods: Samples of 160 formalin-fixed tumors and 17 normal tissues were evaluated by immunohistochemistry for TRAIL-R1 expression. Tumor types included at least 10 samples each of 7 types of carcinoma: colon, breast, pancreas, ovary, prostate, hepatocellular, and non-small cell lung. For neoplasms of the colon, the full continuum of hyperplastic polyp through invasive carcinoma was evaluated. Receptor expression was also studied in 15 other malignancies. Each sample was scored for TRAIL-R1 expression according to intensity of cytoplasmic and membrane staining distribution throughout the tumor.
Results: Of the first 134 malignancies evaluated, 57 (43%) were positive for TRAIL-R1, and 47 were negative (35%). Staining was weak or limited to individual and small clusters of tumor cells in the remaining 30 samples (22%). Thus, a total of 65% of tumors showed some degree of TRAIL-R1 specific staining. TRAIL-R1 staining was consistently weak or absent in all 17 normal tissues. Tumors of the colon, lung and pancreas were most likely to be positive for TRAIL-R1. The level of TRAIL-R1 in the colon was explored further in 26 additional samples representative of typical neoplastic progression. Of these, 10 of 15 adenocarcinomas were positive, while only 1 of 11 benign or focal carcinoma in situ samples were positive. Staining was predominantly membrane and/or cytoplasmic for each individual positive tumor; this pattern, as well as intensity and distribution of positive cells, varied between tumor samples of a single histologic type.
Conclusions: TRAIL-R1 is expressed with variable frequency for different tumor types. Tumors of the colon, lung and pancreas were most likely to express TRAIL-R1. Furthermore, in the colon samples, TRAIL-R1 expression appeared to be increased in malignancies as compared to less advanced neoplasms. All normal tissues evaluated were weak or negative for TRAIL-R1.

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Sulphamoylated 2-substituted oestrogens induce apoptosis through G2-M checkpoint arrest

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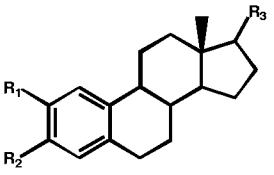
2-Methoxyestradiol (2-MeOE2), an endogenous metabolite of 17β-estradiol, has been found to inhibit cancer cell proliferation *in vitro*, and to inhibit tumour growth *in vivo*. It mediates these effects through microtubule interactions by an oestrogen receptor-independent mechanism. Previously, we have demonstrated that sulphamoylated derivatives of 2-MeOE2 induce p53 expression, cell cycle arrest and apoptosis in human breast cancer cell lines. As an extension of this research, two compounds, 2-MeOE2bisMATE (Compound A) and a D ring modified analogue of compound A (Compound B), were investigated to determine their ability to induce G_2-M cell cycle arrest and apoptosis, at a dose of 1 μM, in a panel of cell lines (A2780, LNCaP, PC3, and MCF-7) using flow cytometric techniques. In all cell lines tested, drug-induced G_2-M cell cycle arrest was seen at 12–18h. In A2780 cells, the percentage of cells arrested in G_2-M were 49% and 47%, treated with Compound A and Compound B respectively. In LNCaP cells, the percentages were, 37% and 33%; in MCF-7 cells, 81% and 37%; and in PC3 cells, 73% and 77%, respectively. However, Annexin V flip-out onto the outer leaflet of the plasma membrane (an indicator of early apoptosis) reveals that the time to resultant apoptosis varies for different cell lines. For A2780 cells, both compounds induced apoptosis at 48h, LNCaP at 72h, MCF-7 at 120h and PC-3 at 96h post-treatment. Western blot analysis of cyclin B1, an essential component of the G_2-M checkpoint whose activity is crucial for progression from prophase to metaphase, indicates upregulation and degradation of this protein occurs at different time points for individual cell lines. The induction of cyclin B1 correlates with entry into mitosis and its degradation with passage through the G_2-M checkpoint and subsequent apoptosis, for all cell lines tested. Taken together, these results suggest that sulphamoylated 2-substituted oestrogens act through induction of G_2-M blockade and bring about apoptosis in cells, which have undergone mitotic slippage or mitotic catastrophe.



Compound	R_1	R_2	R_3
2-MeOE2	MeO	OH	OH
Compound A	MeO	OSO_2NH_2	OSO_2NH_2

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NF-kappa-B: a differential modulator of chemotherapy induced apoptosis

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Members of the NFkB (nuclear factor kappa B) family of transcription factors play key roles in mediating immune, inflammatory and apoptotic responses. Distinct homo and heterodimeric forms are activated in response to a wide range of stimuli including cytokines (e.g. TNF, IL-1), oxidative stress, and DNA damage. Dimers are normally retained in the cytosol in a latent form sequestered by an inhibitor protein (IkB). Despite their diversity, many stimuli lead to the phosphorylation and degradation of IkB allowing nuclear translocation of NFkB and the induction of target genes. Previously we found that a subset of anti-cancer drugs could activate NFkB in cancer cells and that this was dependent on their ability to induce DNA damage. Given the diversity of literature, which has described NFkB's role in both inhibiting and promoting apoptosis, our observations and those of others seem to suggest an anti-apoptotic function in response to DNA damaging chemotherapy. We generated a stably transfected cell-line using cancer cells (MDA-MB-435) expressing an NFkB-GFP responsive promoter. Using this model we confirmed that a range of topoisomerase-targeted drugs (doxorubicin, mitoxantrone, camptothecin) could stimulate the expression of GFP, a marker of NFkB activation, using both Western blotting and immunofluorescent detection. This correlated with IkB degradation and NFkB activation, the latter revealed by EMSA analysis using radioactive labelled NFkB oligonucleotide. However, closer examination revealed distinct patterns of NFkB sub-unit activation, which may reflect the differential response of these cells to the apoptotic potential of individual topoisomerase-targeted drugs. This was determined by supershift analysis and ELISA based detection. Finally, using dual immunofluorescence the temporal pattern of NFkB activation has been analysed in conjunction with Annexin V binding, a marker of phosphatidylserine expression during the apoptotic process. Analysis of individual cells revealed that strong GFP expression did not correlate with apoptotic induction in response to select drugs. Moreover this pattern of activation was influenced by the presence and absence of p53, as revealed through analysis of samples following drug treatment of the HCT116 somatic knockout cell culture model A recent gene-chip experiment has also revealed several potential NFkB regulated genes in response to doxorubicin, which further highlight the role of NFkB in regulating drug-induced apoptosis.