We therefore investigated if a sub-toxic concentration of α-toxin could enhance cisplatin-induced apoptosis, and also performed studies to elucidate how α-toxin affected signal transduction to cisplatin-induced apoptosis.
Cultured human pulmonary mesothelioma cells (P31wt) and a sub-line with acquired cisplatin resistance (P31res) was treated with cisplatin alone or cisplatin supplemented with a sub-toxic concentration (0.1 mg/L) of α-toxin for 48 h. Cell viability was measured with a fluorescein diacetate-based method and the amount of apoptotic cells were determined with TUNEL-staining. Apoptotic signalling was investigated with caspase activity assays, FACS-analysis and Western blot for detection of pro- and anti-apoptotic protein expression.
α-toxin enhanced the cytotoxic effect of cisplatin in P31wt and P31res cells. α-toxin did not affect the number of apoptotic cells in P31wt cells and had only a minor effect on apoptosis of P31res cells. When combined with 5 mg/L cisplatin, α-toxin increased the amount of apoptotic cells from 60 to 75% in P31wt cells and from 25 to 60% in P31res cells. Caspase-3 and -9 were expressed in α-toxin-enhanced cisplatin-induced apoptosis. The pro-apoptotic protein SAPK/JNK was activated by cisplatin in P31wt cells and in P31res cells when combining cisplatin and α-toxin, but not with cisplatin alone.
We conclude that α-toxin significantly increases cisplatin-induced apoptosis in cisplatin-resistant pulmonary mesothelioma cells in vitro. These findings may lead to novel therapeutic strategies to circumvent cisplatin resistance in the treatment of lung cancer.

355 POSTER

Combination therapy with sorafenib and radiation demonstrated improved survival in normal murine gut

F. Herrera[1], S.J. Lunt[2], A. Fyles[1], M. Milosevic[1], S. Supiot[1], J. Ran[2], H. Zhao[2], R. Bristow[2], R.P. Hill[2]. *[1]Princess Margaret Hospital University Health Network, Department of Radiation Oncology, Toronto ON, Canada; [2]Ontario Cancer Institute, University Health Network, Toronto ON, Canada*

Introduction: Sorafenib and Gleevec are small molecules that inhibit signaling through multiple receptor tyrosine kinases (RTK). Sorafenib inhibits signaling through RAF, VEGFR2 and PDGFRb, whereas Gleevec selectively targets Abl, c-Kit and PDGFRa/b. Both drugs have been shown to enhance response to radiation (RT) in experimental models, and sorafenib has been shown to elicit an anti-proliferative and anti-angiogenic effect. We are conducting a series of phase I-II studies with biological targeted therapies in combination with RT in cervix cancer. Intestinal toxicity occurs in a substantial portion of patients who receive standard treatment for cervix cancer. This pre-clinical study was undertaken to evaluate the potential for these novel targeted therapies to increase acute intestinal toxicity further, which would detract from any therapeutic benefit.
Materials and Methods: The intestinal crypt survival assay was used to examine the toxicity of combination therapy with either drug and RT in C3H and Nude mice. Mice were treated with sorafenib (30 or 50 mg/kg/day) or Gleevec (50 mg/kg) for 5 days prior to a single whole body RT dose of 12, 14, 16 or 18 Gy. Control mice received vehicle. Mice were sacrificed 3 days later and the intestines removed and fixed for H&E staining. The numbers of surviving crypts, determined through evidence of mitosis, were counted (blinded) by two independent investigators.
Results: Combination treatment with sorafenib and RT exerted a significant ($p \leqslant 0.05$) radioprotective effect compared with RT alone in both mouse strains. In C3H mice the dose to achieve 10% crypt survival was increased from 15 Gy in control animals to 18 Gy in sorafenib treated mice (ER 1.2). Similarly in nude mice 10% crypt survival was seen at 18 Gy in sorafenib treated mice compared to 13 Gy in controls (ER 1.4). Gleevec did not increased RT toxicity, with both treatment groups showing 10% crypt survival at 16 Gy in C3H mice and 14 Gy in nude mice.
Conclusions: Combined treatment with RT and sorafenib appears to exert a radioprotective effect on normal intestinal crypts relative to RT alone. Interestingly, Gleevec did not have any effect on toxicity. The molecular mechanisms underlying this observation are currently being investigated. Multiple RTK targets can have different effects on the normal gut, and the crypt survival assay may allow rational selection of combination of RTK inhibitors and RT. We will shortly embark on a phase I/II clinical trial of sorafenib in combination with RT and chemotherapy for locally-advanced cervix cancer. The results of this study suggest that RT and sorafenib can safely be combined to treat cervix cancer, with no expectation of increased acute intestinal toxicity.

356 POSTER

Quantitative prediction of therapeutic potential of cancer drugs including pharmacokinetic interactions for apoptosis in 5 min

T. Ona, A. Kosaihira. *Kyushu University, Graduate School of Bioresource and Bioenvironmental Sciences, Fukuoka, Japan*

Recently, National Cancer Institutes (USA) provides the public service of in silico screening to help the efficacy prediction of newly developed cancer drugs. However, in vitro rapid cell-based assay is demanded to verify the prediction quantitatively since a cancer patient may have unconventional aspects of tumor development. Conventional cell survival measurement is time-consuming and most of the cell-based assays are based tedious labeling. Here, we show the rapid and non-label quantitative verifying method and instrumentation of apoptosis via mitochondrial pathway for cancer drugs by the cell reaction analysis of living pancreatic cancer cell cultured on a sensor chip using a high sensitive surface plasmon resonance (SPR) sensor. The time-course cell reaction as the SPR angle change rate for 5 min from 35 min cell culture with a drug was significantly related to conventional apoptosis ratio after 48 h. The results obtained are universally valid with $P < 0.001$ ($n = 63$) for various cancer drugs using various pancreatic cancer cell lines, which mean to contain different level of receptor expression and protein mutation or existence similar to individual patients. Furthermore, they included the pharmacokinetic interactions of drugs which often enhances therapeutic potentials of individually used drugs. The detected SPR signal was derived from the decrease in mitochondrial membrane potential relating to apoptosis via mitochondrial pathway by using carbonyl cyanide 3-chlorophenylhydrazone of specific protonophore for mitochondrial membrane and an apoptotic specific inhibitor of mitochondrial membrane potential through the inhibition of voltage-dependent anion channel, BH4 domain of Bcl-2 family fused to the protein transduction domain of HIV TAT protein. In conclusion, we established a rapid and non-label cell-based quantitative screening method and instrumentation of apoptosis via mitochondrial pathway involving potential targets for cancer drug candidates even designed to target specific cell-signaling pathways. Our system towards the application to evaluate custom therapeutic potentials of drugs including pharmacokinetic interactions for apoptosis using live cells sampled from patients in clinical use.

357 POSTER

The combined effect of non-selective cyclooxygenase-2 inhibitor indomethacin and 5-fluorouracil treatment on colorectal cancer cell lines and xenografts

A. Réti[1], B. Budai[1], V. Komlósi[1], V. Adleff[1], A. Zalatnai[2], A. Jeney[2], J. Kralovánszky[1]. *[1]National Institute of Oncology, Clinical Research, Budapest, Hungary; [2]Semmelweis University, 1st Institute of Pathology and Experimental Cancer Research, Budapest, Hungary*

Background: The high COX-2 enzyme expression in tumours is an unfavourable prognostic factor, however, the influence of COX-2 protein expression levels on tumour response to chemotherapy has been relatively little studied.
The aim of the study was to investigate the effect of 5-fluorouracil (5-FU) combined with the non-selective cyclooxygenase-2 (COX-2) inhibitor, indomethacin (INDO), on HT-29 and HCA-7 human colorectal cancer cell lines and on HCA-7 and HT-29 xenografts bearing SCID mice.
Materials and Methods: Sulphorhodamine B proliferation assay was used to measure the effect of 48 h 5-FU±INDO treatment on HT-29 (low COX-2 protein level) and HCA-7 (high COX-2 protein level) cells. Both cell lines were analysed by Western blot for COX-2 protein levels and by ELISA method for PGE2 production. COX-2 positivity of HCA-7 xenografts was confirmed with IHC. Tumour volume and weight of HCA-7 and HT-29 xenograft bearing SCID mice treated with 6 mg/kg 5-FU s.c. for 5 days ± 2.5 mg/kg INDO p.o. for 20 days were measured. Control mice received vehicle s.c. or 2.5 mg INDO p.o. for 20 days.
Results: 5-FU+INDO treatment compared to 5-FU alone resulted in an enhanced proliferation inhibition on HCA-7 cells ($p = 0.0082$). In contrary, on HT-29 cells no similar effect was observed. After 48 h treatment of HCA-7 cells with 5-FU, INDO or 5-FU+INDO the PGE2 levels were decreased below the detection limit.
Compared to 5-FU treatment the 5-FU+INDO combination caused a significant decrease of relative tumour volume ($p = 0.0236$) and weight ($p = 0.0081$) on HCA-7 xenografts.
COX-2 protein expression of HCA-7 xenografts was markedly reduced after treatment with 5FU+ INDO compared to 5-FU alone.
In contrast, on HT-29 xenografts the 5-FU+INDO combination did not decrease the relative tumour volume and weight. The COX-2 protein levels in HT-29 xenografts were undetectable.