significantly affect their biological activity, as the SEG/Pas-derived Fas/FasL system drives cell apoptosis to a significantly higher extent than the C57BL/6J-system in vitro, and is far more efficient in vivo, subsequently leading to a significant increase in gamma-radiation induced-apoptosis of thymic T cells.
These results lead us to propose that germ-line functional polymorphisms affecting either the levels of expression and/or the biological activity of both Fas and FasL genes could be contributing to the genetic risk to develop T-cell lymphoblastic lymphomas.

351 POSTER

Genetic background and cervical development: the influence of cytochromes P450IID6 genotypes

C. Rodrigues[1], D. Pinto[1], R. Catarino[1], D. Pereira[2], J. Moutinho[3], R. Medeiros[1]. *[1]Portuguese Institute of Oncology, Molecular Oncology Group, Porto, Portugal; [2]Portuguese Institute of Oncology, Medical Oncology Department, Porto, Portugal; [3]Portuguese Institute of Oncology, Gynecology Service, Porto, Portugal*

Background: CYP2D6, a member of the Cytochromes P450 (CYP) family, is a phase I metabolic enzyme involved in the oxidative metabolism of numerous endogenous and exogenous molecules, including pro-carcinogens molecules. The CYP2D6*4 polymorphism has been reported to be a major cause of CYP2D6 poor metaboliser phenotype, leading to the absence or decrease in the amount and activity of its protein. The aim of this study was to understand the role of CYP2D6 genotypes on the development of cervical cancer.
Material and Methods: This study included 378 patients diagnosed with cervical cancer in the Portuguese Institute of Oncology – Porto, Portugal and 334 women without history of oncology disease. DNA was extracted from peripheral blood and submitted to Polymerase Chain Reaction (PCR) followed by Restriction Fragment Length Polymorphism (RFLP), in order to identify the CYP2D6 genotypes.
Results: The genotypes frequencies of the patients group were: 74.30% GG, 21.4% AG and 4.2% AA. In the other group the genotypes frequencies were: 65.57% GG, 27.35% AG and 7.19% AA. We observed that patients carrying the A allele have protection to the development of cervical cancer (OR = 0.698; 95% CI 0.480−1.014; p = 0.059).
Conclusions: The A allele of this polymorphism is responsible of the poor metaboliser phenotype. Therefore, women with AG or AA phenotype will have less ability for metabolizing the pro-carcinogenic molecules, which justify the protective association found in this study to the development of cervical cancer. Our results suggests the influence of genetic background as a cofactor in cervical cancer, a human papillomavirus associated neoplasia.

352 POSTER

Apicidin overcomes TRAIL-resistance on Bcr-Abl expressing K562 cells through inhibition of PI3K/AKT mediated pathway

S.J. Park[1], M.J. Kim[1], H.S. Song[2], H.B. Kim[1], C.D. Kang[1], S.H. Kim[1]. *[1]Pusan National University School of Medicine Pusan National University BK21 Medical Science Education Center, Biochemistry, Busan, Korea; [2]Pusan National University School of Medicine, Biochemistry, Busan, Korea*

Background: Tumor necrosis (TNF)-related apoptosis-inducing ligand (TRAIL) is a pro-apoptotic cytokine that is capable of inducing apoptosis in a wide variety of cancer cells but not in normal cells. Although many cancer cells are sensitive to TRAIL-induced apoptosis, Chronic myeloid leukemia (CML) develop resistance to TRAIL. Histone deacetylase (HDAC) inhibitors are emerging as a new class of anticancer agents, here we investigated histone deacetylase inhibitor apicidin can overcome the TRAIL resistance in CML cells.
Materials and Methods: The effect of combination of apicidin with TRAIL in CML-derived K562 cells was assessed by annexin V analysis. Also, activation of caspase and the changes in the amounts of DR4, DR5, PI3K NF-κB, Bcl-xL, and Bcr-Abl proteins were analyzed by immunoblots. The blocking of TRAIL receptor on apicidin-induced sensitization to TRAIL was evaluated as using neutralizing antibodies DR4 and DR5. The effects of inhibiting PI3K and AKT were also examined by treating K562 with LY294002 and AKT inhibitor IV, which are selective inhibitors of PI3K and AKT, respectively. To explore whether expression of Bcr-Abl contributes to TRAIL-resistance, the sensitization of TRAIL on the Bcr-Abl deleted K562 cells was examined.
Results: Apicidin enhanced TRAIL-induced apoptosis via caspase activation without mediating through TRAIL receptors, DR4 and DR5, although both receptors are expressed in K562 cells. Apicidin downregulated PI3K and enhanced the effect of LY294002 and AKT inhibitor IV on TRAIL induced-apoptosis. Moreover, Bcr-Abl as well as NF-κB and Bcl-xL were also decreased after treating with apicidin, and Bcr-Abl-deleted K562 cells were sensitized to TRAIL.
Conclusion: Our results demonstrated that apicidin can overcome resistance to TRAIL through downregulation of Bcr-Abl and inhibition of PI3K/AKT in K562 cells. Moreover, Inhibition of PI3K activity by apicidin resulted in diminished phosphorylated AKT, inhibition of NF-KB transcriptional activity and significant reduction of expression of NF-KB-dependent protein, Bcl-xL. These were associated with enhancement of the intrinsic sensitivity of cancer cells to cytotoxic effect of TRAIL, therefore, combination of apicidin with TRAIL may be an effective strategy for treating TRAIL-resistant Bcr-Abl expressing CML cells

353 POSTER

Effect of sulfinosine [(R,S)-2-amino-9-beta-D-ribofuranosylpurine-6-sulfinamide] on lung carcinoma cell lines and its role in overcoming multidrug resistance

T. Andjelkovic, M. Pesic, J. Bankovic, N. Tanic, S. Ruzdijic. *Institute for Biological Research "Sinisa Stankovic", Department of Neurobiology, Belgrade, Serbia*

The acquired multidrug resistance (MDR) phenotype in cancer cells is defined as resistance to an applied drug, as well as to many structurally and functionally unrelated compounds. It often develops as a result of changes in drug influx/efflux pumps and changes in glutathione (GSH) detoxification system.
Our research was focused on studying the molecular mechanisms underlying MDR in the non-small cell lung carcinoma cell line (NSCLC) that was selected for resistance to doxorubicin (DOX). In an attempt to successfully modulate MDR, we studied the cytotoxicity of sulfinosine (a guanosine analog) on NSCLC cells, its effect on GSH level in cells and potential to alter the expression of MDR-related genes: mdr1, gst-pi and topo II alpha. In addition, we examined the effects of sulfinosine (SF) in combination with an anti-neoplastic agent curcumin.
The cytotoxic effects of SF, curcumin and their combination on sensitive (NCI-H460) and resistant (NCI-H460/R) cell lines was measured by the sulforhodamine B assay, and their interaction was analyzed with Calcusyn software. GSH level in these cells, both treated and untreated, was assessed using Glutathione Colorimetric Detection Kit. The expression of MDR-related genes was evaluated by semi-quantitative RT-PCR.
Our study showed that the cytotoxic effect of SF was dose-dependent in both cell lines. Interaction of SF and curcumin antagonized growth inhibition in the NCI-H460 cell line while their effect on NCI-H460/R was synergistic. In the NCI-H460/R compared to the NCI-H460 cell line, MDR-related genes had significantly altered expression: mdr1 and gst-pi were 7-fold and 50% increased, respectively, whereas topo II alpha was 2-fold decreased. RT-PCR gene expression analysis in the resistant cell line demonstrated that: (i) SF down regulated the expression level of mdr1; (ii) curcumin decreased the expression level of mdr1 and gst-pi; (iii) a combination of these drugs synergistically decreased the expression of mdr1 and not the expression of gst-pi mRNA.
Further studies revealed that GSH level didn't differ between these two cell lines. Still, it was significantly decreased under low concentration of SF both in NCI-H460 and NCI-H460/R.
In general, our results revealed that the MDR phenotype can be modulated by SF and curcumin, both on level of gene expression and on glutathione level. Moreover, the combined application of these two drugs exceeds the effects obtained after treatment of NCI-H460/R with only one agent.

354 POSTER

A-toxin enhancement of cisplatin-induced apoptosis in cisplatin-resistant mesothelioma cells

D. Johansson[1], K. Grankvist[1], A. Johansson[2], R. Henriksson[3], P. Behnam-Motlagh[4]. *[1]Umeå University, Department of Medical Biosciences Clinical Chemistry, Umeå, Sweden; [2]Umeå University, Department of Odontology Oral Cell Biology, Umeå, Sweden; [3]Umeå University, Department of Radiation Sciences Oncology, Umeå, Sweden; [4]Umeå University, Department of Medical Biosciences Clinical Chemistry and Department of Radiation Sciences Oncology, Umeå, Sweden*

Cisplatin (cis-diamminedichloroplatinum) is a drug used in the treatment of several solid tumors and is extensively used in the treatment of pulmonary mesothelioma. After an initial response the effectiveness of cisplatin is often hampered by inherent or acquired cisplatin resistance causing a severe problem in the treatment of these malignancies. α-toxin (α-hemolysin) from Staphylococcus aureus is a pore-forming toxin which induces apoptosis (intrinsic cell suicide) in eukaryotic cells. Disability to enter apoptosis is a key component in the development of cancer. Finding methods to overcome tumour cell drug resistance to apoptosis could greatly enhance current chemotherapy.

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.