nucleus. Western blotting experiments further verified the fusion protein expression. Histological staining of transfected cells revealed cellular staining. Nuclear staining in transfected cells are significant and probably results from the nuclear localization sequences. FACS analyses suggest that the fusion proteins are secreted and then taken up by the other cells. nlsCre is not secreted or taken up on its own. However when it is fused to VP22 and TAT, they facilitate it's trafficking into the cells.
Conclusion: Amplification of the transgene transmission by using protein transduction domains may provide an opportunity to overcome the limitations caused by the low transfer efficiency of the gene therapy vectors.

1069 POSTER

Three-dimension cell culture and comparison of morphology of four different glioma cell lines

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Background: The characters of individual tissues are determined by constituent cells and this may especially be true in malignant tissues such as brain tumor, because the tissue consists of relatively homogenous population of aberrant cells. On this understanding, cell culture has been utilized for comprehension of the nature of malignant tissues. However, most of such studies were conducted by ordinary monolayer cultures and vital cell functions that are present in living tissues might be overlooked in two-dimension culture. From this viewpoint, we devised a three-dimensional culture that mimics the local environment within the human body. We applied the method to human glioma cells and investigated their properties.
Materials and Methods: Bio-adaptable gelatin was used as scaffold for the three-dimensional culture. Malignant glioma cell lines, T98G, KNS42, A172, and U118MG, were dispersed (1×10^4 cells/100 μl of DMEM), attached to 5-mm cubes of the scaffold, and then further cultivated. The specimens were evaluated morphologically including scanning and transmission electron microscopic examinations.
Results: Glioma cell lines cultured by the method presented distinct features that were hardly detectable in conventional culture. The cells attached to scaffold with extracellular materials and steric cell-to-cell connections were observed throughout the culture. When four glioma cell lines were compared, these lines presented utterly different appearances. U118MG cells tightly attached to the scaffold and dispersed with numerous fibers. In contrast, KNS42 and A172 cells aggregated, clung in each other, and built balloon-like structures. While both cells conglomerated, KNS42 cells bonded more tightly than A172 cells. T98G cells demonstrated intermediate character.
Conclusions: All the glioma cell lines tested grew vigorously by the current culture method. There were whole wide differences between two- and three-dimensional cultures. Four glioma cell lines used for the study were representatives of standard gliomas. Although these cells are frequently used for many culture experiments, their natures were quite different. This became evident only after using our culture. Based on the results, we conclude that our culture method is useful for detailed characterization of gliomas in the human body.

1070 POSTER

Dietary prevention of colon cancer: phytochemical protection of DNA damage and induction of DNA repair in colonocytes

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Colorectal cancer (CRC) is one of the main causes of cancer related mortality in the western world. This disease is a multi-step process involving mutations in critical genes required for maintaining cellular homeostasis. DNA damage can lead to carcinogenesis if replication proceeds without proper repair. Some scientific evidences show that altering dietary habits is an effective and cost-efficient approach for reducing cancer risk and for modifying the biological behavior of tumors. Sage (*Salvia* sp.) plants are rich in many bioactive compounds and may have medicinal properties, such as anticancer activity. In this study, we evaluated the effects of *Salvia officinalis* water extract (SO) and some of its phenolic constituents, rosmarinic acid (RA), Luteolin (Lut), Luteolin-7-glucoside (Lut-7-G) and ursolic acid (UA), a triterpenoid acid, on DNA protection and repair in colon cells (primary cultures of rat colonocytes isolated from *in vivo* treated animals and the human colon cancer cell line Caco-2) exposed to H_2O_2. The comet assay was used to measure DNA damage. Sage water extract and isolated compounds at tested concentrations did not cause damage in Caco-2. RA protected DNA from damage induced by H_2O_2. SO, UA, Lut and Lut-7-G increased the rate of repair (rejoining strand breaks) in Caco-2. *In vivo* treatment with SO also protected DNA damage induced *in vitro* by H_2O_2 in isolated rat colonocytes.
Repair of oxidative damaged bases in all organisms occurs primarily via the DNA base excision repair (BER) pathway. In this study, we also measured the incision activity of a cell extract (Caco-2 cells treated 24 h with SO and isolated compounds) on a DNA substrate containing specific damage (8-oxoGua), to evaluate induction of BER activity. SO, UA and Lut-7-G have a BER inductive effect because they increase incision activity in Caco-2 cells.
In conclusion, SO and the isolated compounds demonstrated chemopreventive activity protecting colon cells against oxidative DNA damage (RA) and stimulating DNA repair (SO, UA, and Lut-7-G).
Acknowledgements: AAR is supported by the FCT, grant SFRH/BD/35672/2007.

1071 POSTER

***Nigella sativa* L. oil ameliorates methotrexate-induced intestinal toxicity through antioxidant activity**

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Background: The efficacy of methotrexate (MTX), a chemotherapeutic agent, is often limited by side effects which were shown to be via oxidative stress. In this study, *Nigella sativa* L (*N .sativa)* oil, a natural antioxidant, was studied as a protective agent against MTX-induced intestinal toxicity via its antioxidant activity.
Materials and Methods: Twenty-four male albino rats were randomly divided into four groups as follows: group (1) saline control, group (2) *N. sativa* oil (10 ml/kg), group (3) saline interrupted on day six by MTX (20 mg/Kg, ip single dose) and group (4) was given *N. sativa* oil and MTX on day six. In the two groups injected with MTX, blood samples were collected at time intervals (0, 1, 3, 4, 5 and 24 hours) to determine serum MTX levels. On day ten, blood samples were collected for hematological assessment of hemoglobin (Hb %), RBCs, WBCs and platelets. All rats were then sacrificed; sections from intestine and liver were cut and homogenized for biochemical analysis measuring measuring glutathione (GSH) content and superoxide dismutase (SOD) activity. Also, sections from intestine, liver and kidney were removed for pathological examination after staining with (H & E).
Results: *N. sativa* oil pretreatment improved food consumption, body weakness and diarrhea caused by MTX. Body weight loss in *N. sativa* oil plus MTX treated group compared to MTX group was (12.7% versus 29.4%, $P < 0.05$). Moreover, severe degeneration of the intestinal mucosa, liver parenchyma, glomerular, and tubular epithelium observed in MTX-treated group were improved by *N. sativa* oil treatment. Parallel to these results, *N. sativa* oil showed significant decrease in SOD content which was elevated by MTX ($P < 0.05$). Whereas, GSH content in MTX group was decreased by 53% compared with those of MTX plus N. sativa oil group ($P < 0.05$). Moreover, addition of N. sativa oil did not significantly change MTX level ($P > 0.05$) excluding interaction. Furthermore, *N. sativa* oil increased total RBCs, WBCs as well as Hb% significantly ($P < 0.05$) compared to MTX but did not cause significant change in platelet count ($P > 0.05$).
Conclusion: Administration of *N. sativa* oil before and after MTX injection ameliorated MTX-induced gastrointestinal toxicity and maintained mucosal structure through anti-oxidant activity. These results can lead to further clinical applications for prevention as *N. sativa* may be used for MTX-induced toxicities.

1072 POSTER

Purification and characterization of a monocot lectin having potent anti-proliferative effect on human cancer cell lines

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Background: Lectins are defined as carbohydrate binding proteins other that enzymes and antibodies. Lectins have emerged as very important macromolecular tools to recognize carbohydrates on cell surfaces. The present work is designed to purify and characterize monocot lectins with interesting biological properties from Indian monocot plants.
Material and Methods: On the basis of sugar specificity determined by hemagglutination, asialofetuin-linked affinity was used to purify monocot lectins. Lectin was characterized for its molecular mass and charge properties by using SDS-PAGE and isoelectric focusing respectively. Standard parameters were used to test the effect of temperature, pH, metal ions and chelating agents. Structural study of lectin was carried out

using fluorescence and CD spectroscopy. Anti-proliferative potential was determined using sulphorhodamine-B assays.
Results: *Arisaema utile* lectin (AUL) gave a single band in SDS-PAGE at pH 8.3 corresponding to subunit Mr 13.5 kDa. The native molecular mass of 54 kDa suggested a homotetrameric structure. Like other monocot lectins, AUL gave multiple bands in isoelectric focusing and in native PAGE at pH 8.3. AUL was inhibited by N-acetyl-D-lactosamine (LacNAc), a disaccharide and asialofetuin, a complex desialylated serum glycoprotein. When treated with denaturing agents, the lectin was stable in the presence of urea (3 M), thiourea (4 M) and guanidine HCl (4 M). The lectin had no requirement for divalent metal ions i.e. Ca^{2+} and $Mn^{2+}$for its activity. AUL was a glycoprotein with a carbohydrate content of 1.2%. Amino acid modification studies of AUL revealed the involvement of tryptophan and tyrosine residues involved in lectin-sugar interaction. AUL exhibited a fluorescence emission maximum (λ_{max}) at 340 nm upon excitation at 295 nm. Using Far UV CD spectra the estimated secondary structure was 37% α-helix, 25% β-sheet and 38% random contributions. *In vitro* anti-proliferative activity of AUL was tested on eleven different human cancer cell lines viz. MCF-7 (Breast), SK-N-SH (CNS), 502713 (Colon), Colo-205 (Colon), HCT-15 (Colon), HT-29 (Colon), SW-620 (Colon), Hep-2 (Liver), IMR-32 (Neuroblastoma), DU-145 (Prostate) and PC-3 (Prostate). The concentrations of AUL which produced 50% inhibition (IC_{50}) of cancer cell lines viz. SW-620, HCT-15, SK-N-SH, IMR-32, Colo-205 and HT-29 at 38, 42, 43, 49, 50 and 89 μg/ml. respectively.
Conclusion: The purified *Arisaema utile* lectin was found to be a homotetrameric protein with potent anti-proliferative effect on human cancer cell lines.

1073 POSTER

The relation between the change of functional cardiac parameters and single nucleotide polymorphisms in Glutathione S transferase P1 and Carbonyl reductase3 genes after doxorubicin chemotherapy

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Background: Glutathione S transferase P1 (GSTP1) is responsible for the detoxification of doxorubicin (Dox), and carbonyl reductase 3 (CRB3) converts Dox to doxorubicinole. Genetic variants of GSTP1 and CRB3 may be contributory to pharmacokinetic and pharmacodynamic variability of Dox, as well as to the interindividual differences in the toxic side-effects. Aim of this study was to investigate the relationship between genetic polymorphisms of GSRP1 (A313G) and CBR3 (V244M) and cardiotoxic effect of Dox assessed by ECG-gated blood pool SPECT (SPECT) and echocardiography (E).
Materials and Methods: Sixty-eight (61F, 7 M) patients, with normal baseline cardiac function, was included. Chemotherapy combinations contained either Dox or epirubicin as 1st line chemotherapy. Systolic and diastolic cardiac functions were evaluated before and after therapy (mean follow-up:10.4$\pm$4.7 months) using E and SPECT. Left ventricular ejection fraction (EF), peak filling rate (PFR), peak ejection rate (PER), end systolic volume (ESV), end diastolic volume (EDV) values were calculated using SPECT data. GSTP1 and CBR3 polymorphisms were analyzed using TaqMan probes.
Results: The mean received anthracycline dose was 508$\pm$153 mg/m^2 (210–1188). Fifteen patients (28%) received adjuvant radiotherapy over the cardiac region. HER2 antagonists were given in 7 patients after chemotherapy. EF values were significantly decreased after AC with both SPECT and E ($p < 0.01$, $p = 0.043$). In 1 patient EF was below 40% after 7 months at 600 mg/m^2 of Dox ESV ($p = 0.028$) and diastolic parameters; mitral inflow e wave deceleration time ($p < 0.001$), mitral inflow colour propagation ($p = 0.001$), and PFR ($p = 0.038$) deteriorated significantly after therapy. Patients who received HER2 antagonists and radiation to cardiac region, showed higher ESV % change ($p = 0.015$, 0.013) compared to others. AA genotype of GSTP1 revealed higher ESV% increase (AA genotype: 9.4 ml $\pm$10; G allele carriers: 3.09 ml $\pm$ 10) after AC ($p = 0.02$). No statistically significant difference between cardiac parameters and CBR3 polymorphism genotypes were found.
Conclusion: This prospective clinical study showed a significant relationship between GSTP1 polymorphism and ESV change after Dox treatment. In the future we plan to increase the number of the patients of this study.

1074 POSTER

Feature of improvement of hormonal therapy: an action code

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Background: Manipulation of the hormonal environment affects breast tumor growth in many species but only never eliminates the tumor completely. Research on influence of a hormonal background on growth and ER status of mice mammary tumors opens a new variants of hormonal therapy.
Methods: The levels of ER were determined by means of the dextran-coated charcoal technique. The resulting data were analyzed by a saturation curve and a Scatchard plot. Tumor transplantation.
Results: On a mouse model it is established, that growth of a tumor at cyclically changing a hormonal level leads to heterogeneity ER status of mammary tumors that complicates hormone therapy. At the same time, continuous influence of such substances as steroid hormones, reserpine, retinol, chorionic gonadotropin results in reduction of value standard deviation growth of tumors in mice. It is established also that continuous application of estradiol leads to homogenization of the estrogen receptor status of mice mammary tumor. Nevertheless, the moment of occurrence of spontaneous tumors is not adequately studied. The results obtained to date suggest that tumor cells may originate in C3H/Sn mice shortly after pairing. Both the originating of tumors and a breeding equally depends on seasons. I obtained data confirm a greater role of steroid hormones and stress for initialization of tumor formation and modulation of its properties. The ability of tumor cells to adapt for change of surrounding microenvironment answers on a question on a paradoxicality of a hormone therapy. Series of tumor parameters such as the invasiveness, heterogeneity, etc. are a consequence of adaptive properties of a tumor cells. Based on experimental methods of research conclusions which can promote improvement of treatment by means of existing methods are received.
Conclusions: Steroid hormones have many advantages to their choice as a medicine against a cancer: a) simple substances freely getting into cells; b) tumor cells of mammary gland answer to low (physiological) concentration; c) influence on a transcription of genes; d) low cost; e) there are natural analogies of application for reprogramming of cell state. The last explains why the effective remedy against a cancer until now is not found. Reprogramming of cells cannot be carried out by one substance or for one act of actions. Serial influence is a code which demands more time for decoding.

Poster presentations (Mon, 21 Sep, 09:00−12:00)
Translational research

1075 POSTER

Sphingosine kinase 1 inhibition sensitizes hormone-resistant prostate and breast cancer cells to docetaxel

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Background: It has recently been shown that docetaxel chemotherapy is effective in prolonging life in patients with prostate cancer (PCa). We have investigated potential ways of increasing the effectiveness of chemotherapy in this disease. We have previously reported that sphingosine kinase 1 (SphK1) inhibition is a key step in docetaxel-induced apoptosis in hormono-refractory PCa cells (Pchejetski, Golzio et al. 2005) and that pharmacological SphK1 inhibition is chemosensitizing in the docetaxel-resistant androgen-sensitive PCa cells (Pchejetski, Doumerc et al. 2008).
Material and Methods: In this current study we have addressed the mechanism of docetaxel-induced apoptosis of PC-3 PCa and MDA-MB 231 breast cancer (BCa) cells and evaluated the synergetic profile of specific SphK1 inhibitors.
Results: Using both PCa and BCa cells we have first identified SphK1-dependent and -independent components of docetaxel induced apoptosis, where SphK1 inhibition is critical for increased docetaxel efficacy. Furthermore we have shown that SphK1 inhibition by docetaxel is a two-step process involving an initial loss of enzyme activity followed by a decrease in SphK1 gene expression. We have demonstrated that both pharmacological and siRNA-mediated SphK1 inhibition leads to a four-fold decrease in the docetaxel IC50 dose.
Conclusions: This work points out to potential ways of increasing the effectiveness of chemotherapy for prostate and breast cancer by SphK1 inhibition.