

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 its 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.

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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 μ l 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.

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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 H₂O₂. 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 H₂O₂. 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 H₂O₂ 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.

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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.

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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 than 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