$p < 0.01$ at 2 weeks). The NFkB protein was more activated after laparotomy than after CO_2 pneumoperitoneum 6 h subsequent to surgical procedures.
Conclusion: After CO_2 pneumoperitoneum, tumours have less TNFa and MIF expression and less NFkB activity than after laparotomy. This may be associated with less tumour growth, supporting minimal invasive techniques in gastrointestinal oncologic surgery.

1013 POSTER

Effect of Polyprenol on DPAGT1 Expression, P-glycoprotein and E-cadherin in MCF-7 Breast Cancer Cells

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Background: The present results are in favour of the idea that N-glycosylation in cancer cells is limited by Dolichyl Phosphate Cycle (DPC) intermediates and DPAGT1 (Dolichyl-phosphate (UDP-N-acetylglucosamine) N-acetylglucosaminephosphotransferase 1 (GlcNAc-1-P transferase) expression. The aim of the present study is to investigate the effect of polyprenol (PP) which provides a Dolichol Phosphate (DolP) substitute on regulation of Pgp and E-cadherin expression in Doxorubicin resistant MCF-7 breast cancer cells MCF-7/ADR-Res.
Methods: Breast cancer cell lines, MCF-7 and MCF-7/ADR-Res were used. Pol concentration in the culture medium made up 10^{-2}–10^{-6}. Immunohistochemical and Western blotting methods were used to detect the changes in the expression levels of E-cdh, MDR1 and DPAGT1 expression. Intermediates of DPC fractions were analysed by HPLC method.
Results: Overexpression of DPAGT1 was 4-fold higher in MCF-7 and 7-fold higher detected in MCF-7/ADR-Res than in human mammary epithelial cells (HMEC). Resistant MCF-7/ADR cells differ from sensitive ones MCF-7 in E-cdh content lost by 3−4 times. It was caused by dolichol-chain shortening and aberrant N-glycosylation of E-cdh in DPC. The study showed 8.5-fold DPC intermediates decrease in MCF-7/ADR-Res cells and 3.6-fold DPC decrease in MCF-7 cells. Resistant MCF-7/ADR cells differ from sensitive ones MCF-7 in Pgp content by 10−12 times. The investigations demonstrate that the situation can be changed by treatment with DolP and PP. The DolP concentration in MCF-7/ADR cells was returned to the normal level. It was established that DolP in the concentration 10^{-6} M aid 7−9-fold reducing Pgp in membranes of MCF-7/ADR cells. The MCF-7/ADR cells cultivation in medium with PP proceeded to give lowered Pgp content in membranes no over 0.4−0.6%, which amount was consistent with the level of Pgp in MCF-7 cells. Treatment of MCF-7/ADR-Res cells with PP in the concentration 10^{-4} M could overcome DPAGT1 overexpression which leads to regulation of E-cdh and Pgp N-glycosylation.
Conclusions: Dysregulation of DPAGT1 causes disturbances in P-glycoprotein (Pgp) expression in multidrug resistance and loss of E-cadherin (E-cdh) in breast cancer cancer cells. Obtained results indicate that E-cdh loss and noncontrollable accumulation of Pgp, after MDR1 expression in MCF-7/ADR cells can be returned to normal level using modulation of N-glycosylation with DolP substitution. DPAGT1 overexpression in MCF-7/ADR can be overcomed with PP.

1014 POSTER

Influence of Chemotherapy on the Lipid Peroxidation and Antioxidant Status in Patients With Acute Myeloid Leukemia

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Background: Chemotherapeutic agents used in patients with cancer cause to generate the enormous amounts of free radicals associated with cell injury. In this study we assess the effects of chemotherapy regimen on oxidant/antioxidant status in patients with acute myeloid leukemia.
Material and Methods: 38 newly diagnosed patients with acute myeloid leukemia (17 women and 21 men) with mean age 34.05±12.49 years were recruited in this study. All patients received Cytarabine and daunorubicin as chemotherapy regimen. Plasma levels of malondialdehyde (MDA), total antioxidant status (TAS), and the levels of erythrocyte activity of superoxide dismutase (SOD) and glutathione peroxidase (GPx) were determined before chemotherapy and 14 days after chemotherapy with daunorubicin and cytarabine.
Result: Plasma MDA concentrations increased significantly (from 2.68±0.89 nmol/ml to 3.14±1.29 nmol/ml) during the 14 days post-chemotherapy period ($P = 0.04$). Plasma TAS concentrations changed with chemotherapy from 1.09±0.15 mmol/L to 1.02±0.14 mmol/L with $P = 0.005$. Erythrocyte SOD and GPX activity decreased overtime from 1157.24±543.61 U/gHb to 984.01±419.09 U/gHb ($P = 0.04$) and 46.96±13.70 U/gHb to 41.40±6.44 U/gHb ($P = 0.02$) respectively.
Conclusions: In conclusion, we report here that there is an increase in malondialdehyde levels and a decrease in the levels of antioxidant enzymes and total antioxidant status. This suggests that chemotherapy causes these changes as a result of enormous production of reactive oxygen species in the patients with AML. Antioxidant supplementation must be approached with caution because of the probability of reduction the therapeutic efficacy of these cytotoxic drugs.

1015 POSTER

Evaluation of the Role of the Novel Glucose-phosphorylating Enzyme ADP-dependent Glucokinase in Human Tumour Cell Lines Using Zinc Finger Nuclease Gene Knockouts

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Aerobic glycolysis is a well-known hallmark of cancer. ADP-dependent glucokinase (ADPGK) is a novel mammalian glucose-phosphorylating enzyme with the unique ability to utilise ADP as phosphate donor. Mouse rADPGK is a monomeric protein of 54 kDa with high specificity for glucose and a K_m of 96 μM. We have found ADPGK to be highly expressed in normal and cancer tissues and that expression is not regulated by either hypoxia or glucose deprivation. Based on these properties, we hypothesised that ADPGK has a protective role under stress conditions such as hypoxia or low glucose by utilising ADP to prime glycolysis when ATP becomes limiting.
To test this hypothesis, multi-allelic ADPGK knockouts (KOs) were generated in H460 and HCT116 cell lines using CompoZr® zinc finger nucleases. ADPGK was also over-expressed using Gateway® cloning. Glucose consumption and lactate formation were measured by Amplex®Red-coupled fluorescence assays and ATP by luminescence. Proliferation and plating efficiency were determined for cells under normoxia, and clonogenic cell killing under restriction of glucose phosphorylation by HK2 siRNA and short-term anoxia (6 hr). Xenografts (n = 6) were grown from wildtype (WT), KO and over-expressing cells to compare tumour growth, necrosis (H&E) and hypoxic fraction (pimonidazole).
ADPGK-null KOs were selected by western blotting, and gene disruption was validated in all alleles by sequencing across the ZFN cut site. H460 KOs were similar in growth to WT, while HCT116 KO lines showed a small reduction in oxic plating efficiency. For H460, 6 hours of anoxia resulted in 45 and 60% loss of clonogenicity for two KO clones compared to WT (4 expts, $p < 0.01$), whereas knockdown of HK2 with siRNA gave 75% cell killing (2 expts, $p < 0.01$). For HCT116, no significant change in survival was found under anoxia (3 expts), while HK2 knockdown resulted in 45% loss of clonogenicity (1 expt). In H460 ADPGK KO clones, ATP was maintained at WT levels, under either normoxia or anoxia, and glucose consumption/lactate formation under anoxia was unchanged even with HK2 knockdown. Xenografts from ADPGK KO cells showed no differences to the WT lines in growth, necrosis or hypoxic fraction.
In conclusion, ADPGK appears to support cell survival under some circumstances in *vitro* without an effect on glycolytic flux and no obvious effect on tumour growth. Conservation of *ADPGK* in metazoa, and its widespread expression in tumours, may reflect a role unrelated to glycolysis.

1016 POSTER

Are CD133 Positive Cells From Esophagus Ascites Cancer Stem Cells?

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Background: The existence of cancer stem cells (CSCs) in acute lymphatic leukaemia were indicated in 1994 by John Dick et al. for the first time. Although CSCs present only 1% of the tumour, they appear to be the only cells, that are able to generate new tumours. For this, CSCs were discussed as the origin of tumour resistance and metastases. For the time being now CSCs could be isolated and characterized only from solid tumours, although Basak et al. (2009) detected CSCs with specific markers in a NSCLC pleura effusion. In many cases CSCs present CD133 as a surface marker but until now it is not clear which characteristics exhibit these CD133+ cells. Because there is no explicit verification of stemness attribute of CD133+ cells, it is very important to clear this question particularly considering the aspect of metastases.