$p < 0.01$ 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.

Material and Methods: We detected CSCs from an esophagus ascites with the stemgent alkaline phosphatase staining kit II and characterised CD133+ cells by separating Lin- and CD133+ cells with the miltenyi diamond separating kit. These cells have been cultured with the esophagus CSC medium from cellsystem and analysed by determined surface marker (CD133, CD166) with FACS, quantitative gene expression of stem cell transcription factors (Oct3/4, Sox2, Nanog, cMyc) and their behaviour of migration under TGFβ stimulation.
Results: After separation of Lin- CD133+ from an esophagus ascites we cultured the cells under specific condition for CSCs. We could show that the number of CD133+ cells increase over a period of 21 days from 1% up to 7.8%. Furthermore we could demonstrate that 4% of CD133+ also positive for CD166. The gene expression of stem cell markers in CD133+ cells compared to CD133- cells showed an up regulation of different markers e.g. Oct3/4, Nanog. We also obtained differences in migration behaviour.
Conclusion: We not only demonstrated that cells with stemness characteristics exist in ascites, but also isolated and characterized them. These cells exhibit markers like CD133 and CD166 which describe for CSCs. Only in CD133+ cells is an up regulation of specific stem cell transcription factors. These results indicate that CD133+ cells from ascites feature a stem cell potential and maybe play an important role in metastases. This hypothesis is supported by the observance that the CD133+ cells showed different migration behaviour compared to CD133- cells. The presented data not only demonstrated the importance of understanding CSCs but also to develop a clinical treatment.

1017 POSTER

Over Expression of ALDH1 as Stem Cell Marker, Is Associated With Mutated BRCA1 in Breast Carcinomas

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Background: Cancer stem cells (CSC) have been described in a variety of malignancies including breast carcinomas. Among several markers aldehyde dehydrogenase 1 (ALDH1) has been identified as a reliable marker for breast cancer stem cells. Knockdown of BRCA1 in primary breast epithelial cells leads to an increase in cells expressing ALDH1.
Methods: We examined 127 breast carcinomas for expression of prospective CSC markers ALDH1, using by immunohistochemistry and correlated with clinicopathological parameters, also with the status of BRAC1 in breast carcinomas.
Results: Cytoplasmic expression of ALDH1 was significantly higher in aggressive tumours (p-value = 0.023), whereas, no significant association was detected between expression of ALDH1 and other prognostic factors. Comparing the results for both ALDH1 and BRCA1 expression showed a significant inverse association between expression of ALDH1 and BRCA, indicating that reduced BRCA1 was more often seen in breast cancer cells expressing ALDH1 (p-value = 0.044).
Combining the results for these two markers, a total of 24/ 110 (22%) of tumours displayed the ALDH1 +/BRCA1 −/low phenotype, which occurred more frequently in higher grade tumours. (P-value = 0.042).
Conclusion: Taken together, our finding suggests that increased ALDH1 was significantly more frequent in aggressive tumours and significantly correlated with reduced BRCA1 in breast carcinoma. Therefore, ALDH1 positive (cancer stem) cells with mutated BRCA1 phenotype intended to be more aggressive and this may indicate a subset of patients for whom more aggressive adjuvant treatment is appropriate.

1018 POSTER

Delayed Cell Death Associated With Mitotic Catastrophe in Gamma-Irradiated Stem-like Glioma Cells

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Background and Purpose: Stem-like tumour cells are regarded as highly resistant to ionizing radiation (IR). Previous studies have focused on apoptosis early after irradiation, and the apoptosis resistance observed has been attributed to reduced DNA damage or enhanced DNA repair compared to non-stem tumour cells. Here, early and late radioresponse of patient-derived stem-like glioma cells (SLGCs) and differentiated cells directly derived from them were examined for cell death mode and the influence of stem cell-specific growth factors.
Materials and Methods: Primary SLGCs were propagated in serum-free medium with the stem-cell mitogens epidermal growth factor (EGF) and fibroblast growth factor-2 (FGF-2). Differentiation was induced by medium containing serum without EGF and FGF. Radiation sensitivity was evaluated by assessing proliferation, clonogenic survival, apoptosis, and mitotic catastrophe. DNA damage-associated γH2AX as well as p53 and p21 expression were determined by Western blots.
Results: SLGCs failed to apoptose in the first 4 days after irradiation even at high single doses up to 10 Gy, but we observed substantial cell death later than 4 days postirradiation in 3 of 6 SLGC lines treated with 5 or 10 Gy. This delayed cell death was observed in 3 of the 4 SLGC lines with nonfunctional p53, was associated with mitotic catastrophe and occurred via apoptosis. The early apoptosis resistance of the SLGCs was associated with lower γH2AX compared to differentiated cells, but we found that the stem-cell culture cytokines EGF plus FGF-2 strongly reduce γH2AX levels. Nonetheless, γIR-induced apoptosis even correlated with EGF/FGF-induced proliferation and mitotic catastrophe in two p53-deficient SLGC lines examined. In a line containing CD133-positive and -negative stem-like cells, the CD133-positive cells proliferated faster and underwent more γIR-induced mitotic catastrophe.
Conclusions: Our results suggest the importance of delayed apoptosis, associated mitotic catastrophe, and cellular proliferation for γIR-induced death of p53-deficient SLGCs. This may have therapeutic implications. We further show that the stem-cell culture cytokines EGF plus FGF-2 activate DNA repair and thus confound *in vitro* comparisons of DNA damage repair between stem-like and more differentiated tumour cells.

1019 POSTER

CD133-/low HT29 Cells Rapidly Re-express CD133 in Vitro and in Vivo and Show Enhanced Survival Under Physiological Oxygen Conditions

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Background: In primary colorectal carcinomas (CRC) CD133 surface expression has been described to enrich for cells with a cancer stem cell like phenotype. The biomarker, however, is under fear debate and we found CD133 expression to not define tumour-propagating cell populations in established CRC cell lines. However, when we studied the *in vitro* properties of $CD133^{-/low}$ vs. $CD133^{+}$ HT29 subpopulations, $CD133^{+}$ cells showed a significantly higher clonogenic survival than their $CD133^{-/low}$ counterparts. This difference unexpectedly neither correlated with response to treatment nor did it translate to loss of tumorigenic potential *in vivo*. We therefore aimed at identifying the rationale for this *in vitro − in vivo* discrepancy.
Materials and Methods: $CD133^{+}$ and $CD133^{-/low}$ HT29 cells were separated via fluorescence-activated cell sorting and studied *in vitro* and *in vivo*. CD133 protein expression was analyzed by flow cytometry and western blotting directly after sort and in short-term 2-D and 3-D cultures. Xenograft tumour formation was monitored in a limiting dilution approach using as low as 10 cells for subcutaneous injection in NMRI (nu/nu) mice. Selected xenografts were extracted, dissociated and CD133 distribution was recorded.
Results: The $CD133^{-/low}$ HT29 subpopulation frequently showed a slight signal shift in flow cytometry and a dim CD133 protein band in western blot analyses directly after sorting. During both 2-D and 3-D culture of the $CD133^{-/low}$ HT29 population, a rapid and massive increase in CD133 expression was observed resulting in a redistribution of $CD133^{+}$/$CD133^{-/low}$ populations within 19 days of culture. A similar redistribution was seen in xenografts derived from $CD133^{-/low}$ HT29 cells. Because we found HT29 to show enhanced clonogenic survival under physiologic oxygen concentrations, we also analyzed cell survival of the subpopulations at 4% oxygen *in vitro* and interestingly found the survival advantage to be higher for the $CD133^{-/low}$ than for the $CD133^{+}$ subpopulation.
Conclusions: The survival advantage of $CD133^{-/low}$ HT29 under physiological oxygen conditions in parallel to the plasticity in CD133 expression are likely to contribute to the lack of phenotypic difference of $CD133^{+}$ vs. $CD133^{-/low}$ HT29 populations *in vivo*.
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1020 POSTER

Detection of Circulating Tumour Cells (CTCs) in Gastrointestinal Tumours Using Reverse Transcriptase-polymerase Chain Reaction (RT-PCR) Method

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Background: Most cancer deaths are caused by haematogenous metastatic spread and subsequent growth of tumour cells at distant organs.