induced mammary gland tumours are cytokeratin 5 (K5) and cytokeratin 6 (K6) positive.
Conclusions: Taken together these data indicate that long term low level expression of GLI1 induces formation of mammary gland tumours with a basal character and that GLI1 expression affects the mammary gland stem cells. The orphan GPCR Lgr5 is expressed in the basal cell layer of the large mammary ducts and might include the mammary stem cell population.

1010 POSTER

Vitamin D Analogs Improve the Antitumour Activity of 5-fluorouracil in Colon Cancer Model MC38

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Background: Colorectal cancer is the third leading cause of cancer death in the Western word. Epidemiological studies strongly suggest a protective effect of calcitriol (1.25-dihydroxyvitamin D3) against colon neoplasia. Moreover, the experimental research reveals its anticancer properties against this type of cancer. The antitumour activity is observed only in hyper-physiological doses of calcitriol, which can lead to hypercalcemia. For this reason the synthesis of vitamin D analogs has been started in order to obtain compounds with better therapeutic activity. On the basis of previous studies we selected two analogs: PRI-2191 (tacalcitol, 1, 24-$(OH)_2D_3$) and PRI-2205 (5,6-trans calcipotriol), which reveal higher antitumour and lower calcemic activity as well as lower toxicity than calcitriol [1, 2, 3].
Materials and Methods: In the current work, it is presented the influence of vitamin D analogs (coded PRI-2191 and PRI-2205) on antitumour activity of 5-fluorouracil (5-FU) in mice bearing transplantable murine colon cancer MC38. The antitumour effect of combined treatment was evaluated as tumour growth inhibition (TGI), increase in life span of treated mice over control (ILS) or tumour growth delay (TGD). The monitored parameters were body weight and tumour volume, which was calculated using the formula (a^2 x b)/2, where a = shorter tumour diameter in mm and b = longer tumour diameter in mm.
Results: We evaluated the most effective dose and treatment schedule with vitamin D analogs combined with 5-FU simultaneously. These studies revealed that the most effective dose for PRI-2191 is 1 μg/kg/day and for PRI-2205 10 μg/kg/day. The best results were observed, when the analogs were injected subcutaneously, three times a week. The application of PRI-2191 or PRI-2205 improve therapeutic effect of 5-FU. Analysis of TGI and ILS indicated synergy between both compounds. Next, we examined potential ability of vitamin D analogs to prolong 5-FU's activity. In this case the application of analogs was started after ended administration of 5-FU. We observed that both analogs also in such schedule of treatment, delayed tumour growth and prolonged survival time in comparison with cytostatic given alone.
Conclusion: Both vitamin D analogs improve antitumour activity of 5-FU in the colon cancer model. We could conclude that the combined therapy of these analogs and 5-FU might be potentially applied to the clinical use.
This work was supported by Ministry of Science and Higher Education Grants: No. PBZ-MNiI-1/1/2005 "New drugs with specific therapeutic and social values" Task: "Vitamin D Analog (PRI-2191) in combination with anticancer agents. In vitro and in vivo" period of 2006–2009 and No. N N401 014535 "Supporting anticancer therapy of colon cancer by using new vitamin D analogs" period of 2008–2011

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1011 POSTER

Survival of Mice With Erhlich Ascitic Tumour Treated With Ultra-dilutions

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Background: This study evaluated the effectiveness of ultra-dilutions (homeopathic remedies on the scales: Hahnemannian decimal − DH and fifty milesimal − LM) on the survival of animals inoculated with Ehrlich ascitic tumour which is highly aggressive and lethal, as a possible therapy for this disease.
Material and Methods: Forty male Swiss mice, weighing about 28 grams each were inoculated intraperitoneally with 10^3 viable cells of Erhlich ascitic tumour. The animals were divided into four groups randomized of ten each (A, B, C and D). Group A was the untreated control. Animals from other groups received as treatment ultra-diluted homeopathic remedies as FAO (Factors of Self Organization) complex in a blind study: *Antimonium crudum*, *Kali carbonicum*, *Mercurius solubis*, *Sulphur*, *Natrum Muriaticum*, *Aurum metallicum*, *Ammonium Muriaticum*. The ultra-dilutions indicated for groups were as follows: B − 12DH/9DH − 5 hours after 10DH/9DH; C − 11DH/9DH − 5 hours after 10DH/9DH; D − 4LM/2LM − 5 hours after 3LM/2LM. The animals were observed until their death, about the survival time in days. The project was approved by the research ethics committees of the University of Medicine of Marília with Protocol 655/08 and followed the guidelines of the Committee on Care and Use of Laboratory Animal Resources, National Research Council, U.S.A.
Results: In group A, the first death occurred at 18 days and the last at 40 days. All mice had ascites and cachexia. In group B, the first death occurred at 75 days. In group C, the first death occurred at 299 days. In group D, the first death occurred at 146 days. After 570 days from experiment beginning, there were still animals alive and in good general condition, presenting the following percentages of survivors: B (10%), C (50%) and D (50%). These animals were euthanized in a CO_2 (carbon dioxide) chamber followed by a macroscopic necropsy. Results showed the absence of ascites and presence of congestion in organs of these animals, such as liver, spleen and lung. The number of survivors was analyzed by Fisher's exact test, comparing groups of animals treated with the control group. The results demonstrated significant differences between the control group A and treated groups C and D, considering the results of probability of $p < 0.05$.
Conclusion: The ultra-diluted remedies used as FAO complex were effective against Ehrlich ascitic tumour. Animals treated had a survival at least 14 folds greater compared to the control group. This study demonstrates the possibility of using ultra-diluted remedies in the treatment of cancer, requiring further studies to exploit these impressive results.

1012 POSTER

Activation of Nuclear Factor Kappa B and Induction of Migrationinhibitory Factor in Tumours by Surgical Stress of Laparotomy Versus Carbon Dioxide Pneumoperitoneum in a Nude Mice Model

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Background: Surgery is the most effective method for the treatment of malignant tumours. However, surgical trauma seems to be associated with enhanced incidence of tumour growth and establishment. At the same time, the mechanisms by which surgical trauma may have an impact on tumour growth and progression still are unclear. Laparoscopic surgery, accepted as a minimally invasive procedure, recently has been adapted for gastrointestinal cancers. Although few clinical studies have shown the oncologic feasibility of laparoscopic surgery, several animal studies mostly have shown that laparoscopic procedures are associated with significantly less increase in tumour growth and metastasis than open surgery. However, a more precise conception regarding the ability of laparoscopic techniques to treat malignant tumours still is needed. A few animal and clinical studies have evaluated the induction of adhesion molecules, inflammatory response, cytokines, and growth factors such as TNFa and vascular endothelial growth factor (VEGF) after laparoscopic and open surgery. These factors may act as indicators for the extent of surgical stress and may modify the biologic activity of dormant cancer cells after surgery. However, these factors have not been evaluated in the tumours after surgery. The authors studied the effect of carbon dioxide (CO2) pneumoperitoneum versus laparotomy on tumour necrosis factor-a (TNFa), migration inhibitory factor (MIF) expression, and nuclear factor kappa B (NFkB) activity in human gastric cancer.
Methods: Nude mice were inoculated intraperitoneally with human gastric cancer cells (MKN45). Then laparotomy, CO_2 pneumoperitoneum, and anesthesia alone were performed randomly. Tumour growth and associated TNFa and MIF expression and NFkB activity were determined.
Results: Total tumour weight, especially at the anterior abdominal wall, was higher after laparotomy than after CO_2 pneumoperitoneum ($p < 0.05$). The mRNA expression of TNFa was higher 24 and 48 h after laparotomy than after CO_2 pneumoperitoneum ($p < 0.05$ and $p < 0.01$, respectively). At all the examined time points, MIF mRNA expression also was higher after laparotomy than after CO_2 pneumoperitoneum ($p < 0.05$ until 1 week or

$p < 0.01$ 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.