status (wild type, deleted, mutated). Western and northern blot analysis were performed to evaluate the protein and mRNA expression of p53, p21, Mdm2, Bax with or without the presence of proteasome inhibitor, MG-132. Transient transfection and luciferase assay was performed to confirm a transcriptional activity of p53. Results: A marked induction of mRNA expressions of Mdm2, Bax and p21 was detected in wild-type p53 expressing cells after the treatment with both B[a]P and 1-NP, but not in either p53-negative or mutant cells. The induced mRNA levels of the p21 did not result in proportional p21 protein increase, indicating the possibility of post transcriptional regulation of the protein. Transcription from the wild-type p21 promoter was markedly induced by PAHs in p53 wild type cells but not in p53 deleted cells. In addition, luciferase activity was not affected by p21 promoter in which p53-binding site is truncated. By the addition of MG-132 to B[a]P treatment, both p21 and p53 protein levels were increased, however, the increase in p21 protein levels was significantly larger than the increase in p53 protein levels. On the other hand, increase in p21 protein was only modest by the addition of MG-132 to 1-NP treatment. B[a]P treatment increased the level of ubiquitinated p21. Cell cycle arrest was more obviously seen by the treatment with 1-NP than by the treatment with B[a]P. Conclusions: These results suggested that the p21 product is degraded by the ubiquitin-proteasome system induced by B[a]P. We conclude that B[a]P-induced p53 protein is transcriptionally active. However, rapid degradation of p21 protein by the ubiquitin-proteasome system may induce a blockade of p53-induced cell cycle arrest, resulting in genomic instability.

468 POSTER

Effectiveness of a new derivative of retinoic acid as differentiating agent on human neuroblastoma cells

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Purpose: Among the compounds that have been explored as differentiating agents, retinoic acid is one of the most potent in the regulation of proliferation and differentiation in neoplastic cells. In the present study we have explored the effect of IIF, (pat.PTC/IT99/00299) a new derivative of retinoic acid, as differentiation inducer in the human neuroblastoma cell line TS12.

Methods: Neuronal differentiation was assessed by means of morphological and cytochemical parameters, i.e. neurite outgrowth, tyrosine hydroxylase (TH) expression and acethylcholinesterase specific activity. The effect of the drug on cell growth was assessed by clonogenic assay.

Results: Treatment with IIF resulted in induction of morphological differentiation, as manifested by the appearance of neurite extension. TH expression was induced by the drug: following RT-PCR on mRNA from neuroblastoma cells, TH mRNA was detectable only in treated cultures but not in control ones. Treatment with IIF induced also a marked increase of acetylcholinesterase activity. Moreover clonogenic efficiency showed the growth inhibitory effect induced by the drug.

Conclusions: These results demonstrate the effectiveness of the new derivative of retinoic acid IIF as differentiation inducing agent on neuroblastoma cell line TS12.

469 POSTER

RBC CR1 in tumour patients: an implication in anaemia?

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Purpose: Tumour patients often suffer from an anaemic condition whose causes are not always completely clear. Increased levels of circulating immune complexes (cIC) are frequently present in these patients. The receptor responsible for cIC clearance is complement receptor 1 (CR1), present also on red blood cells (RBC). The aim of this study was to investigate the presence of RBC CR1 modifications in tumour subjects, as well as the possibility that they are in some way linked to the anaemic condition.

Methods: Patients (age 47-81) affected by breast, lung and colon cancer from stage 1 to 4, were studied; healthy donors, age 19-83, were used as controls. All subjects were submitted to blood withdrawal. Sera were employed for determination of cIC levels by ELISA; RBC were separated from leukocytes, and CR1 expression was evaluated at flow cytometry. The number of CR1+RBC was calculated and correlated to cIC sera levels, subject age and haematological condition.

Results: In tumour patients RBC number was decreased with respect to controls. CR1 expression was also significantly diminished, in a greater proportion than RBC number decrease; on the contrary, cIC levels were significantly increased, especially in the over-60 year old group. The over-60 control subjects also showed increased cIC levels, without CR1+RBC number modifications, and the values were comparable to those found in the under-60 patients. cIC-CR1+RBC correlation was negative in patients, indicating that the CR1 reduction accompanies the cIC increase.

Conclusion: A loss of RBC, in relation with the increased serum cIC level, is suggested as an adjunctive mechanism responsible for both the marked CR1+RBC reduction and anaemia in neoplastic patients; however, the considerable diminution of CR1+RBC (compared with the entire RBC population) also suggests an impaired CR1 production or its augmented proteolysis. CR1 decrease could be involved in the maintenance of high cIC sera levels in these patients (insufficient cIC removal), as well as in the appearance of anaemic condition consequent to the possible elimination from the circulation of cIC-carrying RBC.

470 POSTER

Regulation of vimentin mrna by 12-o-tetradecanoylphorbol 13-acetate (TPA) and all-transretinoic acid (RA) associated with in vitro invasive activity of hep 3b human hepatocellular carcinoma cells

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Purpose: Vimentin is a protein that assembles to form intermediate filaments, one of the major cytoskeletal structures in mammalian cells. Increased expression of vimentin is associated with increasing cancer grade, dedifferentiation, decreased cell-to-cell adhesion, motility, invasion, metastasis, drug resistance and poor prognosis in some cancers. We have reported that the vimentin mRNA was regulated by tumor promoter, TPA and differentiation agent, RA in several cancer cell lines. In this study we found that up- or down-regulation of vimentin mRNA by TPA or RA could modulate the invasive potential of human hepatoma cell line, Hep 3B in vitro.

Methods: To elucidate the role of vimentin gene expression of Hep 3B cells by TPA or RA, we evaluated the vimentin mRNA levels by Northern blot hybridization. Matrix metalloproteinases (MMP-2,-9) and urokinase plasminogen activator (uPA) activities were evaluated using substrate zymography in addition to in vitro invasion assay of Hep 3B cells.

Results: TPA (1-100 nM) treatment showed marked induction of vimentin mRNA up to 48 hrs with a dose- and time-dependent maner and then decreased its level. On the other hand, RA (0.1-10 uM) treatment showed a time-dependent gradual decrease of mRNA level. There was no change of MMP-2, MMP-9 and uPA activities in conditioned medium with concomittent treatment of TPA or RA on zymographic findings. TPA (0.1 uM) treatment significantly enhanced in vitro invasion of Hep 3B cells as much as 2 times, and RA (0.1-10 uM) inhibited the invasion with dose-dependent manner (33.4%, 71.9% respectively) ($p<0.05$).

Conclusion: These results suggest that regulation of vimentin mRNA may be related to invasiveness of Hep 3B human hepatocellular carcinoma cells by controlling cellular motility.

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Inhibition of cathepsin A activity in melanoma cell lines by lactacystin

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Purpose: In recent years considerable attention has been paid to the antitumor activity of the proteasome specific inhibitor, lactacystin. It inhibits the proteasome-mediated degradation of numerous key regulatory proteins which are involved in various cellular processes such as cell division, apoptosis, NF-kB activation, and MHC class I antigen presentation. The ability of the lactacystin to arrest cell cycle progression and induce apoptosis in various tumor cells suggests to their potential use in cancer therapy. Recently we showed, that lactacystin metabolite, b-lacton inhibited the activity

of cathepsin A in vitro, a lysosomal peptidase, which is widely distributed within mammalian cells and tissues, including tumors.

Methods: In the present study we describe the inhibitory effect of the proteasome inhibitor, lactacystin, on cathepsin A activity in murine melanoma cell lines (B16F10, MmB18 and B78) both in vitro and in vivo. Cathepsin A activity was assayed at pH 5.5 using its specific substrate Cbz-Phe-Ala by ninhydrin method.

Results: We have found that lactacystin metabolite, b-lactone, at concentration of 1mM, significantly suppressed cathepsin A activity in B78 melanoma cell lysates by about 50%. Also exposure of three murine melanoma cell lines with different metastatic potential to lactacystin at concentration of 5 mM for 6 hours caused a significant reduction of carboxypeptidase activity of this enzyme, and the inhibitory activity remained unchanged for at least 12 hours. Other proteasome specific inhibitors, e.g. epoxomicin and PSI at concentration of 1mM did not affect cathepsin A activity in melanoma cell line lysates.

Conclusions: The data presented herein support our previous hypothesis that lactacystin is not a specific inhibitor of the proteasome. Since cathepsin A is also tumor-associated enzyme, further research is needed to clarify its role, including the significance of its inhibition by lactacystin, in tumor biology.

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Soluble plasma P-selectin is elevated in patients with advanced carcinoma

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Purpose: Platelet activation occurs in a variety of disease states and is associated with increased levels of both circulating and platelet expressed P-selectin. In cancer patients activated platelets may release angiogenic factors that in turn may promote tumour growth and metastasis. The aim of this study was to investigate platelet expressed and soluble P-selectin levels in patients with advanced and local cancer.

Methods: Twenty-eight subjects were recruited prospectively. Twelve had disseminated malignancy, 6 had locally contained disease and 10 were controls without cancer. Platelet expressed P-selectin (pP-selectin) was measured using a double stained, whole blood, flow cytometry method. Soluble plasma P-selectin (sP-selectin), was investigated using a specific Enzyme Linked Immunosorbent Assay (ELISA) kit, (R&D systems, Minneapolis, Minnesota).

Results: Soluble P-selectin (ng/ml) was elevated in the disseminated malignancy group compared to the locally contained group (66.2 vs. 35.6; $p<0.02$) and the control group (66.2 vs. 38.3; $p<0.05$). There was no statistical difference between the groups for pP-selectin. The platelet count (x109/l) was significantly higher in the disseminated cancer group compared to the control and local cancer groups (337 vs. 212; $p<0.005$ and 337 vs. 253; $p<0.05$ respectively).

Conclusions: Platelet activation occurs in patients with advanced cancer as demonstrated by elevated plasma sP-selectin levels. Therefore, this potential cause for cancer progression should be investigated by further research.

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The effects of intravenous bisphosphonate treatment on the bone microenvironment in patients with breast cancer and bone metastases

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Purpose: Bisphosphonates are potent inhibitors of osteoclast-mediated bone resorption and hence may limit bone-derived growth factors available to breast cancer cells in vivo. This pilot study aimed to investigate the effects of bisphosphonate treatment on cytokines and growth factors in the serum and bone marrow of patients with advanced breast cancer.

Methods: 17 patients with breast cancer and bone metastases were recruited to the study along with 13 patients with primary breast cancer. Samples of serum, urine and bone marrow were taken from the advanced group before and three days after intravenous treatment with pamidronate (90mg) and zoledronate (4mg and 8mg) and from the primary breast cancer group on a single occasion. Serum and bone marrow samples were assayed for the presence of a panel of cytokines and growth factors, including TGFb-1, IGF-1, FGF-2, IL-6 and soluble IL-6 receptor (sIL-6R). Urine samples were assayed for markers of bone resorption.

Results: Samples from patients with advanced breast cancer had significantly higher levels of TGFb-1, IL-6 and soluble IL-6 receptor than those from patients with primary breast cancer ($p<0.05$). In the advanced breast cancer group, no changes were seen in IGF-1, IL-6 or TGFb-1 levels 3 days after bisphosphonate treatment. However, treatment did result in significantly lowered levels of serum FGF-2 and sIL-6R ($p<0.05$).

Conclusion: There appear to be differences in certain cytokines and growth factors between patients with advanced and primary breast cancer. The beneficial effects of bisphosphonates experienced by patients with breast cancer and bone metastases may be due to alterations in cytokines and growth factors, such as FGF-2, that are important in bone. Further studies at different time points are required to confirm these observations.

474 POSTER

Enhancement of WY-14,643-induced transactivation of peroxisome proliferator-activated receptor alpha by green tea extract and its components

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Purpose: Recently green tea was reported to increase the number of peroxisome and its specific enzyme activity in rats. In this study, to determine whether these increases are exerted through activation of peroxisome proliferator-activated receptor alpha(PPARalpha), we investigated the interaction of Wy-14,643, tea extracts, and major tea components with PPAR alpha, cloned from mice using a cell transient transfection assay.

Method: 24 hrs after the transfection, cells were treated with four freshly prepared tea extracts (green tea, oolong tea, black tea, and doongule tea) or tea components in the presence of Wy-14,643. Also, rats received green tea extract (2.5%, W/V) and/or Wy-14,643 (0.5%, W/W) for 2 weeks. Thereafter, RT-PCR was done for acyl-CoA oxidase mRNA.

Result: Activation of PPARalpha was 1.5-2 times increased by green tea extract (0.2%), compared with control. Wy-14,643-induced PPARalpha activation was 4-10 fold enhanced by 0.0001% green tea extract, compared with Wy-14,643 treatment only. Whereas black tea was similar to green tea, semi-fermented oolong tea had little effect on PPARalpha. Even though (-)-epigallocatechin gallate (EGCG) showed the highest activation of PPARalpha and enhancement of Wy-14,643-induced PPAR alpha activation among the components of green tea, its concentration (10 uM) is too higher than expected concentration value of EGCG (<0.9 uM) contained in 0.0001% green tea extract. Oolong tea and doongule tea extract neither increased the activation of PPARalpha nor enhanced Wy-14,643-induced PPARalpha activation. Regarding acyl-CoA oxidase mRNA in the liver of rats fed with Wy-14,643 and/or green tea extract, the combination of Wy-14,643 and green tea extract showed more intensified band compared to that of Wy-14,643 treatment alone.

Conclusion: These results suggest that green tea possess an potent regulatory role in activation of PPAR alpha by peroxisome proliferators, as well as a direct effect through PPAR alpha. In addition, some chemicals like EGCG may have a role in the regulation and be present in the full-fermented black tea.

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BCL-2 down regulation is associated with GO/G1 phase accumulation in 13-CIS-retinoic acid treated HL-60 cells

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Purpose: Retinoic acid and its derivates have been found to cause differentiation of various leukemic cells and therefore are used as a potent therapeutic agent in treatment of acute promyelocytic leukemia. It has been reported that retinoids down regulate the expression of antiapoptotic bcl-2 protein. The aim of this study was to investigate 13 cis retinoic acid regulation of bcl-2 and the possible consequences on cell cycle distribution.

Methods: HL-60 (human promyelocytic cell line) cells were treated with 10-5 M and 10-6 M concentrations of 13-cis-retinoic acid for the period of 24, 48 and 72 hours. DNA content was measured by FACS analysis of propidium iodide stained cells. Cell cycle distribution was estimated using ModFit software. Percentage of bcl-2 protein positive cells was detected