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.

472 POSTER

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.

473 POSTER

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.

475 POSTER

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

by flow cytometric analyses of permeabilized cells tagged with unlabeled primary monoclonal mouse anti-human bcl-2 antibody and with IgG1 FITC conjugated secondary antibody.

Results: Cell cycle distribution data reveale that cells treated with retinoic acid accumulate in G0/G1 phase followed by a decrease in the percentage of cells in S phase in dose dependent manner. The magnitude of change in population of cells in G0/G1 phase and S phase, compared to the untreated controls, was greater after 24 and 48 hours, while after 72 hours cells showed nearly the same cell cycle distribution as 48 hour of treatment.

Percentage of bcl-2 positive cells treated with retinoic acid, expressed as the ratio of treated HL-60 cells vs. untreated controls, showed a dose-dependent decrease in bcl-2 protein expression. During the 72 hour follow-up period, the bcl-2 expression showed maximal decrease at 24 hours which was maintaned at 48 and 72 hours of treatment.

Conclusions: These findings indicate that G0/G1 cell cycle arrest is associated with down regulation of bcl-2 expression possibly due to the up regulation of expression of cdk (cyclin dependant kinase) inhibitors, down regulation of cdks and cyclin B and A levels and hypophosforylation of pRb which prevents synthesis of proteins necessary for the onset of S phase. The observed decrease of the bcl-2 level in retinoid-treated cells could enable apoptotic cell death of differentiated myeloid cells.

476 POSTER

Differential regulation of MMP-9 gene by phorbol ester in "E" and "R" subclones from SW480 human colon cancer cells

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Purpose: The 92 kDa matrix metalloproteinase (gelatinase B, MMP-9) plays a major role in the facilitation of tumor invasion and metastasis. We have reported that isolated "E" type cells from parental SW480 colon cancer cells produced large amount of MMP-9 compared to "R" type cells. In addition, "E" type cells showed much more invasive in vitro and more invasive and metastatic properties in vivo.

Methods: To elucidate the role of tumor promotor 12-0-tetradecanoylphorbol 13-acetate (TPA) on MMP-9 of both subclones, we evaluated the MMP-9 activity and its mRNA level using substrate zymography and RT-PCR. Further evaluation of biological role of MMP-9 regulation by TPA, in vitro invasive ability of both subclones under the influence of TPA was also measured.

Results: MMP-9 activity in the conditioned medium of "E" type cells was markedly stimulated by TPA, whereas the MMP-9 activity of the "R" type cells was refractory to TPA treatment. RT-PCR analysis of MMP-9 mRNA expression reflected the zymographic findings for both subclones. TPA (0.1 nM-1 uM) treatment showed marked increase of MMP-9 mRNA in "E" type cells in a dose-dependent manner, and TPA-mediated stimulation of MMP-9 mRNA expression was blocked by staurosporine, an inhibitor of protein kinase C (PKC). On the contrary, TPA mediated change of the MMP-9 mRNA expression was not found in "R" type cells. Furthermore, 0.1 uM of TPA treatment enhanced in vitro tumor cell invasion of "E" type cells as much as 4.3 times compared to control, and no effect of TPA was found on in vitro invasion of "R" type cells.

Conclusions: These results suggest that differential regulation of MMP-9 in "E" and "R" type cells may be responsible for invasive and metastatic properties of these subclones of parental SW480 human colon cancer cells.

477 POSTER

AP-1 and NF-KB are related to genistein-dependent induction of vimentin gene in HL-60 cells

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Purpose: Genistein is a inhibitor of receptor-dependent tyrosine kinase and vimentin is a growth-regulated gene whose mRNA levels increase after stimulation of quiescent cells. To gain insight on the role of genistein in transcriptional regulation of vimentin gene, the effects of genistein have been investigated in HL-60 cells

Methods: Human promyelocytic leukemia, HL-60 cell line was obtained from the American Type Culture Collection (CCL 240). Total RNA was prepared by a modification of the method of Karlinsey et al. and Northern blot hybridization was assayed by the method of Virca et al. Nuclear extracts were prepared by the method of Lim et al. with a midification of the method of Gorski et al. The binding sites of nuclear protein factors on DNA sequence elements were determined by DNA mobility shift assay.

Results: Genistein induced vimentin mRNA but tyrphostin 25 (T25) and methyl 2,5-dihydroxycinnamate (MDC) had no effect. Genistein increased vimentin mRNA with maximal stimulation reached at 24 hours and the induction of vimentin mRNA was in proportion to concentration of genistein. Increment of vimentin mRNA level by genistein was reduced in the cells treated with cycloheximide or actinomycin-D. In DNA mobility shift assay, one DNA-protein complex of AP-1 and NF-kB was formed when AP-1 or NF-kB binding site was incubated with nuclear extract prepared from HL-60 cells after genistein treatment, respectivety. Genistein-induced AP-1 binding activity was vanished by cycloheximide, but NF-kB binding activity was not changed. Genistein-induced vimentin mRNA was almost reduced by H-7. AP-1 and NF-kB binding activities were also vanished. EGF and PDGF had no effect on genistein-induced vimentin mRNA in HL-60 cells.

Conclusions: Vimentin gene is transcriptionally regulated by genistein in HL-60 cells, and AP-1 and NF-kB may play some role [Supported by the Korean Research Foundation made in the Program year of 1998].

478 POSTER

In vitro/in vivo effects of Taxol on the antitumoral action of irradiation in experimental human tumor model

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Purpose: We have tested the effect of Taxol (Bristol-Myers Squibb) on the antitumoral activity of irradiation in human squamous carcinoma cell lines A431, ECV304 and transformed endothelial cell line, KS-IMM.

Methods: In vitro cultured tumor cells were treated with 2Gy irradiation and/or 7-100 nM Taxol.(10 min.) Cell proliferation was determined by measuring cell densities. Interphase effects on the cytoskeleton was analysed by immunocytochemistry of microtubules and intermediate filaments. Biological consequences were tested in vivo in experimental liver metastasis assay using SCID mice.

Results: None of the treatment schedules had effect on the cell proliferation in vitro. However, profound alterations have been detected in the morphology of cytoskeletal proteins, b-tubulin and cytokeratin analysed by confocal microscopy. Irradiation and low dose Taxol dissaggregated microtubules in interphase cells while high dose Taxol induced severe bundling of microtubules in all the cell lines tested. This later effect was inhibited when Taxol was administered following irradiation. Similar effects were observed in the arrangement of cytokeratin. These data suggested, that low dose irradiation and low/high dose Taxol significantly modulates cytoskeletal structures of interphase cancer cells without affecting cell proliferation. As tumor progression contains several proliferation-independent steps relying on cytoskeletal functions we have tested the effects of the above treatments on the metastatic capacity of the tumor cells. A431 cells were pretreated with low dose radiation and/or low or high dose Taxol in vitro and were injected into the spleen of SCID mice. Animals were terminated 3 weeks later and the weight of the primary tumors as well as the liver metastases were determined. Weight of the primary tumor was not affected by any of the pretreatments. High dose Taxol pretreatment modulated unfavourably the development of liver metastases while low dose irradiation with high dose Taxol inhibited the process. No other treatment regime proved to be modulatory.

Conclusions: These data suggest that Taxol has significant effect on the cytoskeleton of interphase cancer cells, and combination of low dose irradiation and Taxol may have inhibitory effect on metastasis formation, even without affecting the growth of the primary. Our data can be useful in designing new schedules of combined modality treatment of irradiation-sensitive tumors such as head and neck cancer.

479 POSTER

Correlations between Bcl-2, p53 and c-ERB2 proteins expressions in breast cancer: are they determinant in progression evaluation?

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Purpose: Bcl-2, a proto-oncogene originally discovered in a follicular B-cell lymphoma,increase the lifetime of invasive cells by inhibiting apoptosis