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

extending so the cell survival and favour the acquisition and accumulation of genetic alterations; p-53 is a tumor suppressor protein altered in more than 30% of breast cancers; c-ERB-2 oncoprotein overexpression is associated with a poor prognostic in breast cancers. In this context our study tried to establish some correlations between them in order to evaluate the tumor progression.We studied 98 cases with invasive ductal breast carcinomas with tumors T1and T2, immunohistochemical with MoAb.

Material and methods: We studied 98 patients with T1and T2 DICs obtained from two surgical departments between 1995-1999.We used MoAb for Bcl-2 (clone124 Dako A/S,Denmark), p53 protein (clone DO-7 Dako A/S,Denmark) and anti- c-ERB2 (from Boehringer Mannheim), on paraffin embedded tissues. Each case was independently analysed and the number of positive cells was measured in 20 different fields. The cases were evaluated on a specific scale which contain at least three degrees of intensity (except p53 were we measured the IR score).

Results: 65% from tumors with histological grade I expressed Bcl-2 positive cells, and only 12% of them expressed p53.Tumors with histological grade III expressed 34% Bcl-2 positive cells and 56% p53. From the total number of tumors 43,5% overexpressed c-ERB2, 21% from those which were already positive for Bcl-2, and only 43% for p53.

Conclusions: We consider that all these three proeins are very important in evaluating the tumour progression. We noticed a good inverse correlation between tumours which expressed bcl-2 and p53. Anyhow we can observe that c-ERB2 staining is more frequently registered in those tumours which are already altered in p53 expression. Bcl-2 expression is more significant in tumors with histological grades I and II as well as p53. We consider that those two proteins are involved in early events prior to other genetic alterations like c-ERB2 overexpression which is a later step in tumour progression.

480 POSTER

Significance modulation of cell membrane molecules for cell death in hematological cell lines

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Programmed cell death (apoptosis) is essential for normal development and for maintenance of cellular homeostasis in multi-cellular organisms. Apoptosis is regulated tightly by a number of gene products that promote of cell death or extend cell survival, but cytokines produced from NK cell and activated cytotoxic lymphocytes have possibility to induced apoptosis in target tumor cells. TNF as a one from these produced cytokines represents a major mediator of immunlogycal and pathophysiological reactions after engaging appropriated receptors. TNF-α in vitro, either suppresses the growth of some myeloid cell lines or stimulates the growth of some lymphoid cells. Since TNF-α make diverse effects on hematopoietic cells, evaluation of role of some cell membrane surface associated antigen expression on target cells was very important for understanding TNF-α effects following cell death or cell activation process.

In this work we analyzed in vitro TNF-α effects on K-562 (eritroleukemic), Raji (malignant B cell line) and PC (originally developed cell line at Institute of Oncology in Sremska Kamenica) cell line prelabeled with CD45 and CD95 monoclonal antibodies in comparison to untreated cells. Cell were incubated with and without TNF-α in final concentration of 500 and 1000 pg/ml of culture medium for short time period (30 min) and apoptotic and necrotic form of cell death were determined after 2, 4, 6, and 24 h by Flow cytometry (Becton Dickinson). Before and after treatment cell membrane antigen expression were detected on gated cell population. Cell viability was determined by SRB assay simultaneously.

Our results showed that in comparison with untreated cells, TNF-α induced significantly increase in apoptotic and necrotic forms of cell death in Raji and PC cells. The results for apoptotic form of cell death, induced by TNF-α on PC cells pre-labeled with anti CD95 MoAb, correlated with TNF-α effects alone at the same points, while cell death were significantly decreased after 24 h contrary to its effects on Raji cells. In contrast to this, TNF-α induced necrotic forms of cell death between 6 h and 8 h on PC and Raji cells incubated with anti CD45 MoAb, which is a significant increase compared with the effect of TNF-α alone. Results also shows that TNF-α in dose dependent manner significantly decrease cell membrane expression on K-562, Raji and PC cells for evaluated antigens, for more then 2 folds, calculated from basal values expression. Further analyses shows that antigen expression did not correlated with cell death process in all examined cell lines. Decrease of some antigen expression, which partly TNF receptor superfamily members, after TNF-α treatment suggested their in-effectively for induction of apoptotic process but more for activation of the necrotic process. Since modulations of activity TNF-α receptor superfamily was involved in regulation of cell proliferation, cellular activation and differentiation, including control of cell survival or death by apoptosis or necrosis we concluded that CD45 and CD95 participated in transduction of signals from cell surface but with different effects in examined hematopoietic cells.