Conclusion: AP-1 and two novel trans-acting factors (PR & DR) are important to transcriptional regulation of MMP-9 gene during TPA-dependent differentiation of HL-60 cells, and TPA-induced MMP-9 activity may be related to egression of differentiated myeloid cells from bone marrow [This work was supported by grant No. 2000-1-20800-003-2 from the Basic Research Program of the Korea Science & Engineering Foundation].

448 POSTER

Induction of cell transforming activity of benzo(a)pyrene by the glucosinolate gluconasturtiin and phenethyl isothiocyanate extracted from seeds of tide cruciferous *Barbarea verna* in vitro

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Purpose: We assessed the cytotoxic and cell transforming activity of gluconasturtiin (GST), a glucosinolate isolated from seeds of the cruciferous *Barbarea verna*, and its enzyme myrosinase-induced breakdown product phenylethyl isothiocyanate (PEITC). We also assessed whether the presence of GST and PEITC in cell cultures affect the cell transforming potential of the polycyclic aromatic hydrocarbon benzo(a)pyrene (B(a)P).

Methods: An in vitro medium-term (~ 8 weeks) experimental model using BALB/c 3T3 cells was utilized, having as endpoints the formation of cell colonies and cell transformation foci for the cytotoxic and cell transforming activities, respectively.

Results: We found that GST did not exert any cytotoxic activity and was at least one hundred-fold less cytotoxic than PEITC. Furthermore, both GST and PEITC did not exert any cell transforming activity. On the contrary, the presence of both GST and PEITC in cell cultures determined highly significant increases of the cell transforming activity in comparison with that of untreated control (~13-fold and ~ 14-fold higher, respectively), and that exerted by B(a)P *per se* (~ 6-fold and 7-fold higher, respectively).

Conclusions: These findings are at variance with the emphasized cancer prevention potential of glucosinolates and isothiocyanates as modulators of xenobiotics detoxification.. This cocarcinogenic activity of GST and PEITC could be ascribed to the alteration of metabolizing enzymes modulation, e.g. by the enhancement of the presence of phase I xenobiotics metabolizing enzymes, and/or the inhibition of the presence and activity of phase II carcinogens detoxifying enzymes, leading to the formation of cell transforming, active derivatives of B(a)P. Data of our study suggest the necessity of an overall toxicological characterization of any agent before its use on a large scale as cancer preventive agent is recommended.

449 POSTER

The apoptotic effect of TGF-beta in human lymphoma cells

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Deregulated TGF-beta family signaling has been implicated in various human diseases, including autoimmun diseases, vascular disorders and cancers. The growth inhibitory effect of TGF-beta can be blocked at several levels: TGF-beta receptors or downstream signaling mediators (e.g. Smads) could be equally inactivated. Little is known about the loss of TGF-beta sensitivity in B cell lymphomas/leukemias. In normal lymphoid cells TGF-beta have antiproliferative and proapoptotic effects.

Exogenous TGF-beta sensitivity (flow cytometric detection of apoptosis) and the expression of TGF-beta signal transducer molecules (Smad2-4, MAPKs) and their inhibitors (Smad6-7) have been studied in lymphoma cells at RNA (RT-PCR) and protein level (Western blot). The activated exogenous TGF-beta induced apoptosis in HT58, BL41, Daudi Burkitt lymphoma cells, but other cells, Raji, U266 and the isolated human Chronic Lymphoid Leukemia (CLL) cells were resistant to TGF-beta treatment. Signal tranducer Smads are expressed in non-treated HT58 cells as well as in other lymphoma cells (U266, Daudi, Raji, two CLL). Inhibitory Smad6,7 were not expressed in normal peripheral mononuclear cells, but were expressed in the lymphoma cells. Exogenous TGF-beta had no effect on Smad2,3,4 expression, but the expression of inhibitory Smad6 disappeared after 24 hr in HT58 cells. Active JNK and Erk1/2 disappeared rapidly (after 1/2h and 4h) from the cells after TGF-beta treatment in HT58 cells probably via the activation of cellular phosphatases.

These results suggest that both the activation of Smad signals (activating certain apoptotic pathways) and changes in MAPK activities (inhibiting survival factors) are required for the apoptotic effect of TGF-beta.

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450 POSTER

Effect of cytokines on procathepsin D stimulation human breast cancer

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Procathepsin D is via its overexpression and subsequent secretion involved in several types of cancer, mostly breast and ovarian cancer. Our previously published findings demonstrated that procathepsin D serves as an autocrine mitogen. Furthermore we found that the mitogenic activity of procathepsin D is mediated by a receptor different from the currently suggested M6P-R as this reaction was not inhibited by anti-M6P-R or soluble M6P-R. The influence of procathepsin D on tumor cell growth was demonstrated both in vitro using breast and prostate cancer cell lines and in vivo on human breast and prostate tumors in athymic nude mice. Furthermore, these effects seem to be mediated by a structure within the activation peptide of procathepsin D. Based on our experiments, the growth factor activity of procathepsin D can be localized in position 27-44 (and possibly 36-44) of the activation peptide and involves interaction with a new cell surface receptor different from all known M6P receptors. Using monoclonal antibodies raised against individual fragments of the activation peptide we demonstrated strong inhibition of both estradiol- and activation peptide-derived stimulation of breast cancer cell proliferation. In addition, using an in vivo model of human breast cancer, we showed that injection of tumor-bearing mice with biodegradable microspheres containing anti-fragment or anti-activation peptide antibodies inhibited the growth of breast cancer. In addition, IL-4, IL-10 and IL-13 stimulated secretion of procathepsin D in dose dependent manner, addition of anti-procathepsin D antibodies inhibited the interleukin-stimulated cell proliferation. From our results we can hypothesize that with respect of procathepsin D secretion in breast cancer, some interleukins act in similar fashion as estrogens Based on presented experiments, we propose that the interaction of procathepsin D activation peptide with a new surface receptor is mediated by a sequence 36-44 plus close vicinity and leads in certain types of tumors to a potentiated growth and higher motility. The activation peptide is a new potential target for suppression of growth and spreading of several types of tumors including breast tumors.

451 POSTER

The host environment in tumor progression: the liver as a model

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Retrorsine (RS) is a naturally occurring pyrrolizidine alkaloid which exerts a long lasting block on hepatocyte cell cycle. We have shown that transplantation of normal hepatocytes into rats pre-treated with RS results in massive repopulation of the recipient liver by donor-derived cells (Am.J. Pathol. 153:319, 1998; 158:771, 2001). In the present studies, the fate nodular hepatocytes isolated at various stages of carcinogenesis, and transplanted into RS-treated recipients was followed. The dipeptidyl-peptidase type IV-deficient (DPPIV-) rat model for hepatocyte transplantation was used in order to distinguish donor-derived from recipient cells. Liver carcinogenesis was induced in Fischer 344, DPPIV+ rats according to the protocol developed by Solt and Farber. In a first study, livers were perfused 6 months after the initial treatment and grossly visible (>5 mm) nodules were separated from surrounding tissue. Cells isolated from either tissue were then injected (via portal vein) into either normal or RS-treated DPPIV- rats. In RS-treated recipients, transplanted nodular hepatocytes grew rapidly into visible nodules and replaced >90% of the host liver within 2 months, finally progressing to hepatocellular carcinoma within 4 months post-transplantation (Tx). By contrast, no growth of nodular cells was observed in normal-untreated recipients. Furthermore, tumor progression was delayed when initiated cells were transplanted together with surrounding hepatocytes.These results indicate that (i) a growth-constrained host environment is able to drive tumor progression of transplanted nodular hepatocytes; moreover, (ii) shared mechanisms appear to sustain liver repopulation on one hand and tumor progression on the other hand, depending the type of hepatocyte transplanted, normal vs. nodular. Supported by MURST (Italy) and ROTRF (Roche Organ Transpl. Res. Found.)