444 POSTER

Modulation of metastatic potential of v-src transformed cells as a result of exogenous DAP-kinase activity

E. Zueva[1], A. Kimchi[2], A. Tatosyan[3]. [1] *Cancer research Centre, Oncogene Regulation Laboratory, Moscow, Russia;* [2] *Weizmann Institute of Science, Departament of Molecular Genetics and Immunology, Rehovot, Israel;* [3] *Cancer research Centre, Oncogene Regulation Laboratory, Moscow, Russia*

Purpose: In order to identify cellular genes and proteins involved in the metastatic process of transformed cells a panel of cell lines transformed by variants of v-src oncogene with different metastatic potential in vivo were used. Recently discovered DAP-kinase gene (a novel type of calmodulin-dependent serine-threonine kinase with antitumorigenic and antimetastatic effects) was introduced into cells with different metastatic capacity. The activity of selected signal transduction proteins and biological properties of new isolated cells were examined.

Methods: transfection of DAP-kinase containing vector into v-src-transformed hamster fibroblasts with different metastatic potentials. Analysis of experimental (EMA - i.v. injection of the cells) and spontaneous (SMA - s.c. injection of the cells) metastatic characteristics of the transfectants in vivo. Comparison of expression of different signal transduction proteins supposed to be involved in metastatic processes.

Results: DAP-kinase significantly suppressed EMA and SMA of transformed cell lines. Parental and control (carrying empty vector) lines induced from 50 to 300 metastatic nodules in the lungs of all i.v. injected animals. DAP-kinase expressing cells never induced more than 20 nodules. SMA test also shows multiple reductions of metastatic activities of DAP-kinase expressing cells: 60% of these cells were not able to produce metastasis at all, 40% produced 20-100 less nodules in the lungs. The differences in metastatic potential of analyzed cells in vivo was also confirmed in vitro by collagen degradation test: cells producing DAP partially lost collagenase activity i.e. this parameter correlates with suppression of metastatic potential of transformed cells. It was found that production and activity of selected members of signaling pathways (MAP-k, FAK, paxillin, Shc, Grb2 etc) were changed in cells carrying different phenotype after introduction of DAP-kinase gene. Production and enzymatic function of v-src of transfected lines was not changed. The compared spectra of genes expressed in cells with different metastatic status obtained by a cDNA array hybridization technique will be presented.

Conclusion: The described collection of new cells (8 lines) with modulated metastatic properties is a unique model system for identification of specific genetic and molecular factors responsible for invasive and metastatic behavior of tumour cells.

445 POSTER

The role of Irf-1 and caspase-7 In ifn-gamma enhancement ofFAS-mediated apoptosis in achn renal cell carcinoma cells

Y. Tomita, V. Bilim, N. Hara, T. Kasahara, K. Takahashi. *Niigata University Graduate School of Medicine, Urology, Niigata, Japan*

Caspases exist as zymogens, and are activated by various extracellular stimuli, leading to apoptosis. One such stimulus is Fas/CD95, a member of the the tumor necrosis factor receptor family, providing one means of cytotoxic T lymphocyte(CTL) -mediated cell lysis. Clinical evidence has shown that administration of cytokine leads to regression of renal cell carcinomas (RCCs). Interferon-gamma (IFN- g) indicates its contribution to anti-tumor activity of immune cells. IFN- g elicits its effect through the transcription factor signal transducer and activator of transcription-1, (STAT-1), and through interferon regulatory factor-1 (IRF-1), one of the target genes of STAT-1. Our previous study demonstrated an increase in the susceptibility of ACHN cells, established from RCC, to Fas-mediated apoptosis by IFN-g, and the inhibition of this effect by the caspase-3 and -7 inhibitor, DEVD-CHO. In ther present study, we demonstrated the following phenomena in IFN- g -treated ACHN cells: 1. Enhanced transcription of caspase-1, 3 and 7 mRNAs without any change in cleavge of their substrates; 2. Increased cleavge DEVD (specific for caspase-3 and 7), but not YVAD (for caspase-1) or DMQD (for caspase-3), after anti-Fas/CD95 mAb treatment; 3. Activation of the STAT-1 and IRF-1 pathway; 4. Partial abrogation of the IFN- g -induced increase of Fas-mediated apoptosis by antisense IRF-1 oligodeoxynucleotide. These results indicate that IRF-1 plays a pivotal role in the IFN- g-mediated-enhancement of Fas/CD95-mediated apoptosis, through regulation of DEVD-CHO-sensitive caspases, most likely caspase-7.

446 POSTER

Identifying novel p14ARF binding partners

S. Ivanchuk[1,2], S. Mondal[1,2], J. Rutka[1,2]. [1] *The Hospital for Sick Children, The Arthur and Sonia Labatt Brain Tumour Research Center, Toronto, Canada;* [2] *The University of Toronto, Laboratory Medicine and Pathobiology, Toronto, Canada*

Introduction: Biallelic deletion of chromosome 9p occurs in 30-60% of high grade gliomas including glioblastoma multiforme. The INK4A/ARF locus has been mapped to chromosome 9p and encodes two functionally distinct cell cycle inhibitory products arising from alternative splicing of unique first exons to a common second exon. p16INK4A (p16) regulates cell cycle progression through interaction with cyclin-dependent kinases 4 and 6. p14/p19ARF (ARF) is generated from splicing of an upstream first exon to a common exon 2 resulting in a transcript that is read in an Alternative Reading Frame. ARF engages in a tertiary complex with MDM2 and p53 resulting in p53 stabilization and commensurate arrest in G1 and G2.

Purpose: Recently, evidence for a p53-independent mechanism of ARF-mediated growth arrest has been suggested. Reconstitution of ARF was found to elicit growth arrest in p53/MDM2/ARF triple knockout fibroblasts indicating the potential existence of as yet unidentified mediators of ARF activity. In addition, we stably transfected ARF into a human astrocytoma cell line (SF-126) which is deficient for both p53 and ARF activity. Soft agar assays were performed and ARF was found to significantly reduce colony formation upon reconstitution in these cells.

Materials and Methods: In an attempt to determine if novel binding partners are responsible for the p53-independent ARF activity observed, we performed a yeast two-hybrid screen of a human fetal brain cDNA library using full length human ARF fused to GAL4 DNA-binding domain as bait. A total of 1X106 clones were isolated and 12 positive clones identified. BLAST searches were performed on sequences extracted from the positive clones. Three of these positive interactants are nuclear proteins with known functions in cells while a larger percentage are novel, as yet uncharacterized proteins. We are currently characterizing these interactions to determine their relevance in a mammalian system.

Conclusions: We have identified novel binding partners of the ARF protein in mammalian cells. Characterizing these binding interactions will help elucidate ARF's role in cells as well as in tumourigenesis.

447 POSTER

Characterization of trans-acting factors in transcriptional regulation of MMP-9 gene during TPA-dependent differentiation of HL-60 cells

K. Lim[1,3], M.Y. Son[1], Y.K. Kim[1], J.I. Park[1], W.H. Yoon[2,3], S.K. Park[1,3], B.D. Hwang[1,3]. [1] *College of Medicine, Chungnam National University, Department of Biochemistry, Daejeon, Korea;* [3] *Cancer Research Institute*

Purpose: Matrix metalloproteinase-9 (MMP-9) is believed to play a critical role in tumor cell invasion and metastasis, and may also be important to the egression of differentiated myeloid cells from the bone marrow. To gain insight on the mechanism of egression of differentiated myeloid cells from bone marrow, transcriptional regulation of MMP-9 gene expression and invasion during TPA-dependent differentiation of HL-60 cells have been investigated.

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. 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 DNase I footprinting assay and DNA mobility shift assay.

Results: MMP-9 mRNA and activity were increased in Northern blot hybridization and zymographic analysis in time- and dose-dependent manner during TPA-induced differentiation of HL-60, respectively. MMP-9 expression was inhibited by the treatment of protein kinase C inhibitors, H-7 and calphostin C. In DNase I footprinting analysis, the nuclear factors interacting with the five cis-elements were identified in undifferentiated HL-60 cells: unidentified protein (-240), proximal repressor (PR, -445), TRE (-533), SP-1 (-563) and distal repressor (DR, -599). AP-1 binding activity (TRE, -79) was appeared, and PR (-445) and DR (-599) were disappeared in the nuclear extract prepared from TPA-treated HL-60 cells. The trans-acting factors were bound in proportion to the concentration of nuclear extract. In mobility shift assay AP-1 binding activity was induced by the nuclear extract prepared from TPA-treated HL-60 cells and decreased by the treatment of H-7. Motility and invasiveness of HL-60 cells were increased to 30.7 fold and 19.8 fold after the TPA treatment, respectively.

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

P. Perocco, M. Mazzullo, A.M. Ferreri, P. Rocchi. *Department of Experimental Pathology, Cancerology Unit, University of Bologna, Bologna, Italy*

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

A. Sebestyen[1], G. Barna[1], A. Gondor[1], K. Nagy[1], S. Weishede[1], P. Bauer[2], L. Kopper[1]. [1] *Semmelweis University, Ist Department of Pathology and Experimental Cancer Research, Budapest, Hungary;* [2] *Semmelweis University, Department of Biochemistry, Budapest, Hungary*

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.

Supported by OTKA (D29111, T26391), ETT (192/2000, 193/2000)

450 POSTER

Effect of cytokines on procathepsin D stimulation human breast cancer

V. Vetvicka[1], J. Vetvickova[1], Z. Voburka[2], M. Fusek[2]. [1] *University of Louisville, Pathology, Louisville, USA;* [2] *IOCB, Biochemistry, Prague, Czech Republic*

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

S. Laconi, P. Pani, E. Laconi. *Experim. Pathology, Medical Sciences and Biotechnology, Cagliari, Italy*

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.)