444 POSTER

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

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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

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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

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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

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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.