627 POSTER

Novel trans-acting factor, MMP-2-specific sequence binding protein, is related to TGF-b1-dependent regulation of MMP-2 gene in HL-60 cells

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Background: The matrix metalloproteinase (MMPs) are a family of extracellular matrix degrading proteinases, which are reported to play an important role in the metastasis and invasion of a number of human cancers, and may also be important to the egress of differentiated myeloid cells from the bone marrow. To gain insight on the mechanism of egress of myeloid leukemia cells from bone marrow, the invasiveness and the regulation of MMP-2 gene expression by TGF-b 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 modification of the method of Virca et al. Nuclear extracts were prepared by the method of Lim et al. with a modification of the method of Gorski et al. The binding activities of nuclear protein factors on DNA sequence elements were determined by DNase I footprinting and DNA mobility shift assay.

Results: TGF-b1 enhanced the motility and invasiveness of HL-60 cells. TGF-b1 increased MMP-2 and TIMP-2 mRNA levels, whereas MMP-9 and TIMP-1 mRNA were not induced. All TGF-b isotypes (TGF-b1, b2, b3) increased MMP-2 gene expression with the maximum at 12 hr, and the increase was a dose-dependent manner. In DNase I footprinting analysis, one trans-acting factor (MMP-2-specific sequence binding protein, MSSBP) interacting with the region from 37 to 18 bp (MMP-2-specific sequence, MSS) was identified in the nuclear extract prepared from control cells, but MSS was not protected by the nuclear extract prepared from TGF-b1-treated cells. PD98059 (inhibitor of ERK1/2) and SB203580 (inhibitor of p38 MAP kinase) reduced TGF-b1-dependent MMP-2 gene expression in a dose-dependent manner. In DNA mobility shift assay, the reduction of the binding activity on MSS of nuclear extract prepared from TGF-b1-treated HL-60 cells was restored by the pretreatment of PD98059 and SB203580.

Conclusion: The expression of MMP-2 gene is regulated by TGF-b1 and a novel trans-acting factor (MSSBP) may be important to transcriptional repression of MMP-2 gene in HL-60 cells. Moreover, ERK1/2 and p38 MAP kinase is related to signal transduction of TGF-b1-induced MMP-2 gene expression. [This work was supported in part by Korea Research Foundation Grant (KRF-005-D00004)].

628 POSTER

Influence of exogenous PTEN on multidrug resistance of human and rodent cells.

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Background: PTEN the tumor suppressor, inhibits cell growth and induces apoptosis (in unfavorable conditions), its activation can increase sensitivity to chemotherapy of some types of tumor cells, and its inactivation could induce drug resistance. The aim of this study is to elucidate the molecular mechanisms of the influence of PTEN gene on the sensitivity of mammalian cells to chemotherapeutic agents. We studied the influence of PTEN cell sensitivity to drugs with different intracellular targets, on the MDR1 gene expression and on P-glycoprotein (Pgp) activity.

Materials and methods: Cell lines used in the study: Drug sensitive, parental cells: KB 3-1 and MCF-7 human carcinomas, HET-SR-2SC-LNM RSV transformed fibroblasts of the Syrian hamster (2SC). Drug resistant cell lines: 8-5, MCF-7/Adr, 2SC/20-2. Cells were transiently transfected with PTEN vector or control vector and sensitivity of transfected and control cells to adryablastin (ADR) and colchicine (CH) was compared. Pgp functional activity was measured by means of FACScan analysis of Rh123 efflux from the cells. Apoptosis after transfection was studied by flow cytometry analysis of PI stained cells. The expression of MDR1 and PTEN genes were determined by RT-RCP technique; Pgp expression was analyzed by indirect immunofluorescence using monoclonal antibodies UIC2; PTEN expression was studied by Western blotting.

Results: We examined endogenous PTEN expression and Pgp activity in parental and MDR cells. There was no correlation between PTEN expression and the rate of Pgp functional activity. PTEN transfection did not alter Pgp activity and expression. PTEN transfection increased ADR resistance only of MCF-7/Adr cells, all other cell lines became more sensitive to ADR. Almost all examined cell lines acquired resistance to CH after PTEN transfection, however 2SC cells which are intrinsically CH-resistant became more CH sensitive.

PTEN-transfected cell lines proliferated more slowly than the cells transfected by the control vector. Spontaneous apoptosis increased 2-fold after PTEN transfection.

Conclusions: Our data show that PTEN do not influence Pgp expression and functional activity. However PTEN introduction into the cells selected for Pgp-mediated MDR can alter cell sensitivity to the drugs in other way than the sensitivity of drug sensitive cells. Further studies are needed to understand the mechanism of this phenomenon. PTEN increased cell sensitivity to ADR, but induced cell resistance to CH inmost of the cells studied. Probably the influence of PTEN gene on cellular drug sensitivity depends on the mechanism of drug action upon the cells as well as on cell context.

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Localization of beta-catenin and its role in the biological characteristics of oral squamous cell carcinoma cells

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Background: Beta-catenin plays an important role in the Wnt signal. Wnt signal inhibits GSK-3β that is responsible for the phosphorylation of beta-catenin and as consequence, unphosphorylated beta-catenin is increased. Since unphosphorylated beta-catenin is not degradated by proteasome, it accumulates in the cytoplasm. Thereafter, it translocates into the nucleus and interacts with the Tcf/Lef transcription factors activating expression of the target genes such as c-myc and cyclinD1. In the present study, we examined the localization of beta-catenin in the oral squamous cell carcinoma (SCC) cell lines and tissues, and whether Transfection of mutated beta-catenin could modify the biological characteristics of the SCC cells.

Material and methods: Human oral SCC cell lines HSC-3, SAS, Ca9-22 and KB were used. The tumor tissues were obtained from 20 Japanese patients with oral SCC The localization of beta-catenin was examined by immunohistochemistry. Exon3 of beta-catenin gene contains a site that is phosphorylated by GSK-3β. A mutated form of beta-catenin cDNA lacking exon3 was transfected to HSC-3 cells and a permanent cell line was established. The expression of beta-catenin was examined by Western blot analyses and immunohistochemistry. Migration assay was performed using BIOCOAT chamber.

Results: Immunohistochemical staining revealed that beta-catenin localized in both cytoplasmic membrane and cytoplasm of HSC-3, SAS and KB cells, but not in the cytoplasm of Ca9-22 cells. Of 20 cancer tissues, 18 samples (90%) expressed beta-catenin in both cytoplasm and membrane, 2 samples (10%) expressed beta-catenin only in the membrane. Mutated form of beta-catenin was confirmed by Western blot analysis and the accumulation of the beta-catenin in the cytoplasm and especially the nuclei was demonstrated by immunohistochemical staining. Different from the parental cells, the transfected cells showed a cuboidal morphology and loose intercellular contact. The transfectants continued to proliferate after they reached confluence.

Conclusions: In a large proportion of the oral SCCs, the cytoplasmic accumulation of beta-catenin was confirmed. Moreover, expression of beta-catenin in the cytoplasm and nuclei by transfecting mutated beta-catenin caused biological alterations. These results suggested that the cytoplasmic and nuclear accumulation of beta-catenin is associated the enhanced growth and migration of the oral SCC cells.

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Angiotensin Converting Enzyme (ACE)-inhibitor inhibits tumour growth and metastases formation in the Lewis Lung Carcinoma.

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Background: ACE inhibitors (ACEi) have been prescribed for hypertension since 1980 and have only negligible toxicity. They interact with the catalytically active site in ACE, a metalloproteinase. Experimental studies have indicated a matrix metalloproteinase (MMP)-inhibiting effect of ACEi.

The aim of this study was to examine the effect of the ACE inhibitor ramipril (RAM) on tumour growth and metastases as well as the underlying mechanisms.

Material and methods: A murine Lewis Lung tumour and metastasis-model was used. C57BL/6J mice were inoculated subcutaneously with $2x10^5$ tumour cells on day 1. Treatment was initiated the following day as RAM 30mg/kg/day in the drinking water, cyclophosphamide (CTX) 100mg/kg intraperitoneally (i.p.) day 2, doxorubicin (DOX) 2.4mg/kg i.p. day 2-5 or combinations of RAM+CTX or RAM+DOX. Tumour size was recorded three times per week and mice were sacrificed on day 28. The lungs were processed for stereological determination of metastasis volume. Tumours were collected and examined for expression of MMPs at mRNA and protein levels by RT-PCR and Western blot. Some of the lungs were examined for MMP expression at the protein level as well.

Results: Based on time to reach a tumour volume of $800mm^3$ Kaplan-Meier plots were constructed for each treatment group. Compared to saline-treated controls, RAM treatment significantly increased time to tumour volume $800mm^3$ (23 days versus 21 days, $p<0.0001$, log rank test) and significantly decreased the volume of lung metastases ($P=0.002$, Mann-Whitney test). RAM+CTX and RAM+DOX significantly increased time to tumour volume $800mm^3$ (25 days versus 21 days, $p<0.0001$ and 25 days versus 23 days, $p=0.0013$, log rank test). Lung metastasis volumes were also significantly reduced by the combined treatment regimens ($p=0.003$ and $p=0.015$, Mann-Whitney test) compared to treatment with CTX or DOX alone.

MMP-2 and MMP-9 were measured at the mRNA level in tumour extracts and at the protein level in tumour and lung extracts. RAM had no effect on the mRNA level of either MMP. In tumours the MMP-2 and MMP-9 protein expression were similar in all treatment groups. In lungs RAM-treatment tended to increase MMP-2 protein expression and decrease MMP-9 protein expression.

Conclusion: Treatment with RAM significantly inhibited tumour growth and lung metastasis formation. The effect of two different cytostatic agents on these parameters was increased when RAM was added, suggesting an additive or even synergistic effect.

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Nitric oxide up- regulates cyclooxygenase-2 expression through the cAMP- response element in its promoter in a head and neck cancer cell line

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Background: We previously observed the over-expression of cyclooxygenase-2 (COX-2) and the increased production of prostaglandin E by nitric oxide (NO) in several cancer cell lines. In this study, we investigated the mechanisms of interaction between the NO and COX-2 pathways in head and neck cancer cells.

Material and Methods: For our experiments, we used plasmids containing partial COX-2 promoter region and the fusion *trans*-activator plasmids (pFA-ATF-2, pFA2-CREB, and pFA2-cJun from stratagene) and performed western blotting and promoter-luciferase assay.

Results: cAMP-response element (CRE) was identified as a critical factor of COX-2 expression in SNU-1041. It was found that at least three transcription factors (TFs) - CREB, ATF-2 and c-jun, could bind to CRE of the COX-2 promoter and that their activities were increased by SNAP, a NO donor. Also we found that the activation of soluble guanylate cyclase, p38 and JNK by NO might play an important role in COX-2 over-expression through the up-regulation of these three TFs. The effect of dibutyryl-cGMP on COX-2 expression was similar to that of SNAP and was blocked by a p38 inhibitor, not by a JNK inhibitor. In addition, we found that dibutyryl-cGMP might activate CREB and ATF-2, whose activities were increased by p38, but not c-jun. Moreover, NO induced JNK signaling followed by the activation of c-jun and ATF-2 in cGMP-independent manner.

Conclusions: These results imply that NO generated endogenously at low concentrations may affect many gene expressions, including COX-2, which can promote the growth and survival of tumor cells.

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Genomic DNA amplification of Decoy receptor 3 (DcR3) correlates with cancer progression of well-differentiated colorectal adenocarcinoma.

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Background: Decoy receptor 3 (DcR3), a member of tumor necrosis factor receptor (TNFR) superfamily, shows inhibitory effect on Fas- mediated apoptosis. We have reported the relationship between DcR3 mRNA overexpression and the progression of well-differentiated colorectal adenocarcinoma (17th Meeting of the European Association for Cancer Research, abstract #403). In the present study, we examined the relationship between DcR3 gene amplification and Fas mRNA expression, and also examined the correlation of DcR3 gene amplification with apoptotic cell death to clarify its effect(s) in human well- differentiated colorectal adenocarcinoma.

Materials and methods: Tissue specimens were obtained from 27 patients with well-differentiated colorectal adenocarcinoma who underwent operations at Niigata University Hospital between 1998 and 2002. Genomic DNA of cancer tissue was extracted from paraffin embedded sections by microdissection under light microscope. Total cellular RNA, extracted from tissue samples of both cancer and nomal mucosa, were reverse-transcribed to synthesize cDNA. Quantitative real- time PCR was carried out to determine genomic DNA amplification of DcR3 and mRNA expression of Fas by standardizing with b-globin gene. In 19 patients, cancer cell death was examined visibly by in situ enzymatic labeling of DNA strand breaks using Apoptag in situ oligo ligation (ISOL) kit. Statistical analysis was performed by Mann-Whitney U-test, Kruskal- Wallis test, and Spearman's correlation coefficient by the rank test, and the statistical significance was defined as $P<0.05$.

Results: Genomic DNA amplification of DcR3 was found in 23 cases (85.2%), and was significantly increased in patients with tumor invasion deeper than subserosa or non-peritonealized perirectal tissues ($P=0.0014$), and in the vascular invasion-positive patients ($P=0.014$). There was no significant correlation between DcR3 gene amplification and other clinicopathological features including Fas mRNA expression. By ISOL in situ cell death detection assay, DcR3 gene amplification was significantly increased in apoptotic cells-negative patients compared to positive patients ($P=0.0412$).

Conclusions: These results suggest that DcR3 gene amplification in well-differentiated colorectal adenocarcinoma may be one of the factors for evasion of apoptosis, and may be involved in cancer progression effecting variables such as depth of tumor invasion and vascular invasion.

633 POSTER

Specific activation of Akt3 in ovarian cancer

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Background: The serine/threonine protein kinase Akt exists as three isoforms; Akt1, Akt2 and Akt3. Akt, which is activated in response to mitogenic stimuli, may contribute to tumourigenesis at multiple levels with the kinase shown to play prominent roles in several processes considered hallmarks of cancer including the regulation of proliferation, cell survival, invasiveness and angiogenesis. Recent studies suggest deregulation of specific Akt isoforms may be involved in individual tumour types including ovarian, breast and pancreatic cancers. Levels of Akt1 and Akt2 activity have been shown to be amplified in 6 and 36% of primary ovarian tumours, respectively. However, this work has been limited by lack of specific reagents for the Akt isoforms. This study has investigated the role of Akt3, in parallel with Akt1 and Akt2, in ovarian cancer.

Materials and Methods: The expression of each Akt isoform was assessed by western blotting using isoform specific antibodies. Akt activity was determined by direct assay using the specific peptide substrate RPRAATF, by isoform-specific immunoprecipitation assays and by western blotting with phosphospecific antibodies. Expression of Akt2, Akt3 and activated Akt (representing all 3 isoforms) was assessed in primary ovarian tumour samples by immunohistochemistry.

Results: A screen of isoform expression in 8 ovarian cancer cell lines and a non-tumourigenic control cell line, revealed Akt1 expression to vary across the cell lines, whereas Akt2 was detected in only one cell line (OVCAR3). Expression of Akt3 also varied across the cell lines with marked overexpression in 2 of 9 cell lines tested (OVCA429 and DOV13). Total Akt and Akt3 specific activity was shown to correlate with overexpression of