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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)].

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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.