PS 1811 EFFECTS OF VARIOUS METALS ON SECRETION OF MATRIX METALLOPROTEINASE2 FROM MOUSE FIBROBLAST CELLS.

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Several different metal compounds have been identified as carcinogens. Recent study showed that matrix metalloproteinases (MMPs) were secreted enzymes selectively degrading the extracellular matrix and had been implicated in tumor cell invasion. Therefore, we have carried out a study on the effects of a series of metals (Cd^{2+}, Cr^{6+}, Cu^{+}, Fe^{3+}, Ni^{2+} and Zn^{2+}) on secretion of MMP from mouse embryonic fibroblast cells, immortalized by SV40 large T-antigen. In zymographic analysis, high level of 68kDa latent enzyme of MMP2 was secreted in the conditioned medium of the cells. The cells were exposed to the metals for 24 hours and conditioned medium was collected for zymographic analysis. Secretion of MMP2 from the cells was found to be strongly inhibited by several metal ions. Cd^{2+} was the most effective followed by Zn^{2+} and Cr^{6+}. Treatment of 0.1μM or 0.5μM Cd^{2+} depressed the secretion of MMP2 by up to 60% and 30%, respectively. However, Cd^{2+} did not affect MMP2 gene expression. These data indicated that Cd^{2+} inhibited secretion of MMP2 from the cells without decrease of MMP2 synthesis. Invasiveness of the cell to reconstituted basement membrane Matrigel was significantly inhibited by 0.5μM Cd^{2+} for 24 hours. These results indicate that Cd^{2+} inhibits cell invasion by decreasing secretion of MMP2 from cells. Unexpectedly, Cd^{2+} appeared to be an effective anti-metastasis in this system. (Supported by a Grant-in-aid for General Research from the Ministry of Education, Sciences, Sports and Culture of Japan.)

PS 1812 TRANSCRIPTIONAL ACTIVATION OF MTF-1: THE ROLE OF PKC AND CK2 CONSENSUS SITES ON METAL-INDUCIBLE ACTIVITY.

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Metallothioneins (MTs) are low molecular weight, cysteine-rich metal binding proteins that play central roles in metal homeostasis. Metal-inducible MT transcription is regulated by the interaction between the cis regulatory metal responsive element (MREs) and the MRE-binding transcription factor MTF-1 (metal-regulatory transcription factor-1). The mechanism by which metals activate MTF-1/MRE-mediated transcription has not been resolved. We propose a model in which the regulation of MT transcription is controlled by signal transduction cascades that affect MTF-1 phosphorylation, which include PKC and CK2. We investigated the role of potential PKC and CK2 phosphorylation sites using site-directed mutagenesis of MTF-1 and lentiviral transduction of dko7 cells (MTF-1 null murine embryonic fibroblasts). We identified two PKC consensus sites, T224 and S641, where mutation of the phosphate-accepting residue to alanine resulted in defective MT transcription. S641 is also a potential CK2 phosphorylation site. A previous mutagenesis study of murine MTF-1 using a heterologous yeast expression system showed that a mutation of the T241 CK2 consensus site to alanine also produced a loss-of-function (lof) phenotype. In the present study, dko7 cells transduced with a T241A mutant had defects in cadmium-inducible MT transcription. To investigate the role of phosphorylation in MTF-1 activation, we transduced dko7 cells with phospho-mimetic aspartic acid residue at T224 or S641 and examined changes in MT transcription. qPCR analysis demonstrated that the phospho-mimetic mutations did not rescue the lof phenotype of the T224A and S641A mutations. In addition, these mutations inhibited MT transcription, compared to dko7 cells expressing wild type mMTF-1 in the absence of metal. These results suggest that MT transcription is controlled by a change in the phosphorylation pattern of MTF-1 that may also include dephosphorylation.

PS 1813 REGULATION OF CELL DIFFERENTIATION BY METALLOTHIONEIN-3 (MT-3) IN HUMAN PROXIMAL TUBULE CELLS.

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Cadmium (Cd^{+2}), a heavy metal and well-known nephrotoxicant is known to cause kidney disease through inflammation and necrosis of the proximal tubule cells. Metallothioneins, a group of low molecular weight, cysteine rich, intracellular proteins is known to sequester heavy metals such as Cd^{+2} and mercury and provide protection against their toxic effects. Previous studies from our laboratory have shown that third isoform of metallothionein (MT-3), a unique member of metallothionein family is involved in the maintenance of vectorial active ion transport in cultures of human proximal tubule (HPT) cells. We also demonstrated that MT-3 is involved in regulation of cell differentiation by controlling the epithelial-to-mesenchymal transition (EMT) and mesenchymal-to-epithelial transition (MET). Recent *in vivo* and *in vitro* studies have reported that MT-3 is interacting with other proteins and these interactions are thought to be playing an important role in execution of some of its functions. The goal of our study is to identify the binding partners of MT-3, which may allow us to understand the mechanism through which MT-3 is regulating the vectorial active transport and cell differentiation in HPT cells. We performed MT-3 pull-down experiments followed by SDS-PAGE and mass spectrometry analysis in an immortalized human proximal tubule cell line, HK-2 cell extract and renal cortical tissue extract. We have identified β-actin, tropomyosin, gelsolin and myosin (non-muscle) as the binding partners of MT-3 and our immunofluorescence studies have shown that MT-3 is co-localizing with the above mentioned proteins. These studies demonstrate that MT-3 is interacting with the proteins that are involved in cytoskeleton reorganization of the cell and thereby regulating the vectorial active transport and cell differentiation.

PS 1814 THE CARBOXYL-TERMINAL CYSTEINE RESIDUES OF MTF1 ARE CRITICAL FOR ARSENIC SENSING AND INDUCTION OF MT1.

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Metal-activated transcription factor 1 (MTF1) mediates the induction of metallothioneins (MT) I and II by zinc and stress signals. The mechanism of MTF1 activation has not been well understood. We analyzed the interaction between arsenic (As) and MTF1 for Mt1 induction. As potently induces Mt1 mRNA expression in mouse hepa1c1c7 cells. Induction is dependent upon functional MTF1 as induction is lost in Mtf1 knockout (KO) cells but is restored upon reconstitution with Mtf1; Moreover, As induces the binding of MTF1 to the metal response elements of endogenous Mt1. Induction is not affected by modulating zinc concentrations but is markedly enhanced by cycloheximide. Phenylarsine oxide (PAO) that covalently binds to vicinal protein cysteine thiol groups induces Mt1 with a magnitude of higher potency than that of As. PAO affinity beads effectively pulls down the carboxyl half of MTF1 (MTF1 321-675) by binding to a cluster of five cysteine residues near the terminus. Preincubation with As, Cd, Co, Ni, Ag, Hg, and Bi blocks pull-down of MTF1 321-675 by PAO beads in vitro and in vivo, indicating binding of the metal inducers to the same C-terminal cysteine cluster as PAO. Deletion of the C-terminal cysteine cluster or mutation of the cysteine residues abolishes or markedly reduces the transcription activation activity of MTF1 and the ability of MTF1 to restore Mt1 induction in Mtf1 KO cells. The findings demonstrate a critical role of the C-terminal cysteine cluster of MTF1 in arsenic sensing and gene transcription via arsenic-cysteine thiol interaction.

PS 1815 TWO PKC CONSENSUS SITES IN MTF-1 REGULATE ITS TRANSCRIPTIONAL ACTIVITY.

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Metallothioneins (MTs) belong to a superfamily of intracellular proteins that sequester environmentally toxic metals and are thought to regulate their intracellular concentration. MT expression is transcriptionally regulated by the metal-regulatory transcription factor 1 (MTF-1). MTF-1 acts by binding to short DNA sequences called metal responsive elements (MREs) found in the enhancer/promoter regions of target genes including MT-I and MT-II. To examine the role of phosphorylation in MTF-1 transcriptional activation, PKC consensus sites were identified within MTF-1 and each site was systematically mutated. Lentiviruses were developed for each mutant, transduced into MTF-1 knockout mouse embryonic fibroblasts (dko7 cells) and stable cell lines expressing each construct were isolated. We exposed these cells to cadmium or zinc, and measured changes in expression of two marker genes: MT-I and MT-II, by real time qPCR. dko7 cells transduced with wild type MTF-1 demonstrated significant levels of metal-inducible transcription of the two marker genes examined. However, the MTF-1-T224A and MTF-1-S641A mutants did not show metal-inducible transcription of MT-I and MT-II. Surprisingly, an MTF-1 mutant containing mutations in all 6 PKC consensus sites yielded levels of MT-I and MT-II metal induction similar to cells transduced with wild-type MTF-1 after exposure to cadmium or zinc. Consistent with this finding, whole cell extracts prepared from cells expressing wild-type MTF-1 and the MTF-1 mutant protein harboring all 6 PKC mutations were able to bind MREs sequences in electrophoretic mobility shift assays; but, cell extracts containing MTF-1-T224A or MTF-1-S641 mutant proteins did not show MREs binding. These data support the idea that phosphorylation regulates the ability of the transcription factor to carry out inducible transactivation of MT genes.