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### Gene Expression by Zinc Deficiency under Homocysteine Level in EA.hy926 Cell Using cDNA Microarrays

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Hyperhomocysteinemia is a known risk factor for atherogenesis and zinc deficiency has been shown to increase circulating levels of homocysteine in rats. What we hypothesized was that zinc deficiency would increase the harmful effects of homocysteine on endothelial cells, and molecular markers of homocysteine-related changes by zinc level would be needed. In this study, we determined the zinc-regulated gene expressions under high and low homocysteine level in EA.hy926 cells, which were derived from umbilical vein cells, using cDNA microarray. EA hy926 cells were cultured in Zn-deficient media(Zn-, 0 $\mu$M Zn) containing chelexed fetal bovine serum or in Zn-adequate media (Zn+, 12 $\mu$M Zn as $ZnCl_2$) under each high homocysteine (Hcy+, 10 mM) or low homocysteine (Hcy-, 0 mM) level, respectively. After 3d treatment, cells were harvested and total RNA was labeled with Cy3 or Cy-5 to obtain labeled cDNA probe. Fluorescent dye was swapped for assessment of dye effect. Fluorescent labeled cDNA probe was applied to microarray slide(human 1K) for hybridization, and then the slide was scanned using fluorescence scanner. Gene spots were analyzed on the basis of four experimental replications. In Zn+, comparing to Zn-, under Hcy+, upregulated 6 genes were mostly related with intracellular modulators, and downregulated 1 gene was transcription protein($p<0.05$). In Zn+ under Hcy-, 5 genes which were mostly about transcription related protein and cytokines were upregulated, and 6 genes in which apoptosis-associated proteins and cytokines were included, were downregulated($p<0.05$). Zn effect in Hcy- would be interest of the further study due to the downregulated genes of apoptosis and cytokines for clarifying molecular zinc aspect in atherosclerosis.

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### Zinc Assessment Using Mononuclear Cells Metallothionein mRNA Expression by Competitive-Reverse Transcriptase-Polymerase Chain Reaction (c-RT-PCR) in Human Subjects

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Marginal Zn deficiency is prevalent through the whole world and, yet, it has not been develpted to assess biological Zn level in human subject due to the lack of a reliable diagnostic indicator. One potential possibility for zinc assessment using Zn-binding protein, metallothionein(MT)-mRNA has been proposed. The purpose of the present study was aimed to show whether measurement of mononuclear cells(MNCs) MT mRNA, using a c-RT-PCR assay, could indicate Zn status in human subjects. In the this study, MNCs MT-mRNA expression was measured using c-RT-PCR to compare before(baseline) and after(supplement) 5 day of Zn supplement period (50 mg Zn/d as zinc gluconate). Mononuclear cells were purified from venous blood using Histopaque. RT-PCR oligonucleotide primers which were designed to amplify both a 278 bp segment of the human MT cDNA and a 198 bp bp mutant competitor cDNA template MT-mRNA were normalized by housekeeping gene to $\beta$-actin mRNA which was also measured by c-RT-PCR There was considerable inter-individual variation in MT-mRNA concentration and, yet, the mean level after Zn repletion period was increased, comparing to the baseline Zn level. This MT-mRNA level was shown as the same pattern of the conventional plasma and RBCs Zn assessment. in which plasma Zn level was increased ($p=0.02$) and RBCs Zn level was also increased after Zn supplement($p=0.18$). We may suggest that MT c-RT-PCR can be a more useful assessment assay of evaluting of Zn status.