GS2-1 **Mechanisms of cross talk among copper related proteins in chaperone "Atox1" and metallothionein defected mouse fibroblast**

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Copper (Cu) is one of the essential elements and also highly toxic, thus, cells elaborate the Cu homeostasis. However, the mechanisms underlying the Cu regulation in cells are not fully understood. In the present study, the knockdown on one of the Cu chaperone genes, Atox1 was introduced into mouse fibroblast established from metallothionein (MT)-null cells. The aim of this study is to reveal the MT-independent detoxification mechanisms for Cu using MT-knockout cells bearing Atox1-knockdown with multi-disciplinal techniques. The multi-disciplinal techniques consisted of two specific techniques for metallomics. First, speciation was used to show the chemical species of Cu in soluble fraction of cells. As the sample from cultured cells were too small to analyze by the conventional HPLC-ICP-MS, a micro HPLC-ICP-MS system was adopted. Second, a Cu fluorescent probe, copper sensor 1 (CS1) was used. This probe offers the visualization of intracellular Cu distribution in living cells. The intracellular Cu concentration was increased with Atox1-KD in MT-KO cells, and elevated Cu was compartmentalized in cellular vesicles. Although the intracellular Cu concentration was actually elevated, the several manifestations indicating the Cu deficiency appeared in the cells. These suggest that Cu compartmentalized with the vesicles may be less bioavailable than Cu bound to MT. Our results provide new insights into the mechanisms involved in maintaining the Cu homeostasis, namely, novel roles of the cellular vesicles and MT in the detoxification and utilization of Cu in mammalian cells.