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## 銅シャペロンAtox1ノックダウン/MTノックアウト細胞における銅制御因子の発現

○宮山　貴光[1], 小椋　康光[1], Dennis　Thiele[2], 鈴木　和夫[1]([1]千葉大院薬, [2]Duke University Medical Center)

【Introduction】Copper (Cu) is one of the essential elements. Because free Cu has high toxicity, its homeostasis is strictly regulated in mammalian cells. However, Cu regulated system is incompletely characterized. In the present study, Atox1 gene was specifically knocked down with small interfering RNA (siRNA) in mouse metallothionein knockout (MT-null) cells. The effect of RNAi in the cells was evaluated by Cu concentration and distribution, and Cu related gene expression analysis.

【Methods】Mouse MT-null cells were transfected with or without siRNA targeting Atox1 for 24 hr. Gene expression levels of Cu transporter (Ctr1) and chaperones (Atox1, Ccs and Cox17) were quantitated by real time RT-PCR and Western blotting. A 5 μL portion of the supernatant of cell lysate was applied to narrow bore HPLC-ICP-MS for Cu speciation, and Cu concentration was quantitated by ICP-MS combined micro flow nebulizer. Using Cu sensor 1 (CS1), the knockdown cells were subjected to Cu distribution analysis.

【Results and Discussion】As the results of RT-PCR and Western blotting, Ctr1 mRNA and proteins were paradoxically increased in Atox1 knockdown MT null cells, and intracellular Cu concentration was increased comparing to control cells. However, although SOD1 expression level was constant, Ccs levels were elevated. These observations suggest that the increased Cu observed in Atox1 knockdown MT null cells is not bio-available because it is known that Ccs levels increase in response to low Cu. Experiments with CS1 Cu-fluorescent imaging suggests that the Cu in these cells may be compartmentalized in cytoplasm. These results suggest that Cu-related gene from the reason of MT knockout showed Cu deficient-like phenomenon despite Cu excess induced Atox1 knockdown.