Metallothionein and Zinc Dynamics in Tumor Cells Using Inductively Coupled Plasma Mass Spectrometry

David H. Petering, Christy L. Kiekenbush, Susan K. Krezoski, and Frank E. Laib

University of Wisconsin – Milwaukee; Dept. Chemistry/Biochemistry

Tumors and other proliferative tissues display a rigorous requirement for nutrient Zn. Numerous tumors and cultured cell lines also contain large concentrations of metallothionein (MT). Recently, in a number of examples, direct correlations have been made between tumor pathogenicity and MT concentration. Experiments with cultured Ehrlich cells have demonstrated that under conditions of extracellular Zn-deficiency, Zn is rapidly mobilized from Zn-MT in a process that is not rate limited by protein turnover. It is hypothesized that MT-bound Zn was transferred to other sites through ligand substitution processes. Present studies have investigated the nature of the involvement of MT in tumor cell Zn metabolism under steady state, Zn-normal conditions. In particular, the hypothesis has been examined that Zn-MT provides a labile Zn-buffer or intermediate in the trafficking of Zn^{2+} between its point of entry into TE671 glioblastoma cells and various Zn-proteins. Cells were incubated with medium containing 8 ìM of the stable isotope ^{67}Zn until they were uniformly labeled. Then, at time 0 the cells were transferred into the same growth medium containing 8 ìM ^{66}Zn. Time dependent changes in the ^{67}Zn and ^{66}Zn content of the MT and aggregate higher molecular weight steady state conditions of the experiment, the cells express metal saturated Zn-MT that binds 10% of the cytosolic Zn. Cellular uptake and incorporation of ^{66}Zn was driven by the expansion of the dividing cell population (doubling time 24 h). The MT Zn pool rapidly exchanged ^{67}Zn for ^{66}Zn with a $t_{1/2}$ of 1.5 h. A small fraction of ^{67}Zn remained in the protein over 24 h, perhaps indicating a recycling of metal ion released upon biodegradation of the ^{67}Zn–protein pool. The aggregate high molecular weight (HMW) proteins, also acquired ^{66}Zn over time with 60% occurring with a $t_{1/2}$ of 25 h, indicative of a dilution of the ^{67}Zn isotope as additional cells were made in the ^{66}Zn medium, assuming that internal Zn did not exchange with extracellular Zn but instead was recycled during protein degradation and synthesis. It was found that ^{67}Zn-cells did not release significant ^{67}Zn into Zn-free Hanks medium or into the medium containing ^{66}Zn over a 4 h period. These results support the hypothesis that the observed changes in the cellular Zn isotope content did not result from ^{66}Zn-^{67}Zn exchange processes. The findings confirm the cellular lability of Zn in MT and suggest a possible role of MT in Zn-trafficking. Further, they indicate that the dynamics of intracellular Zn metabolism can be examined with stable isotope methods. Supported by NIH-ES-04026 and ES-04184.