Zinc homeostasis in myeloid cells is regulated by epigenetic mechanisms

Zinc is an essential trace element with integral roles in numerous biological processes, including enzymatic function, protein structure, and cell signaling pathways. This metal serves as a catalytic or structural cofactor for about 300 different proteins. Abnormal zinc homeostasis causes a variety of health limitations that include neuronal dysfunctions, growth retardation, hypogonadism, and immunodeficiency.

Zinc deficiency has adverse effects on immune cell functions. Therefore, maintaining a constant state of cellular zinc nutrition resp. zinc homeostasis is essential for normal function and an efficient immune response. The uptake of zinc into cells and its transport into and out of intracellular organelles is regulated by fourteen human importing (ZIP, solute-linked carrier (SLC) SLC39A; Zrt/IRT-like protein), and ten exporting (ZnT, SLC30A) zinc transporters. Furthermore, zinc binding proteins metallothionein (MT) and S100A8/S100A9 control intracellular zinc levels through buffering excess zinc or supplying zinc under zinc deficiency. Epigenetics describes the transmission of properties which do not involve changes in the underlying DNA sequences but rather inherited changes in gene regulation and gene expression.

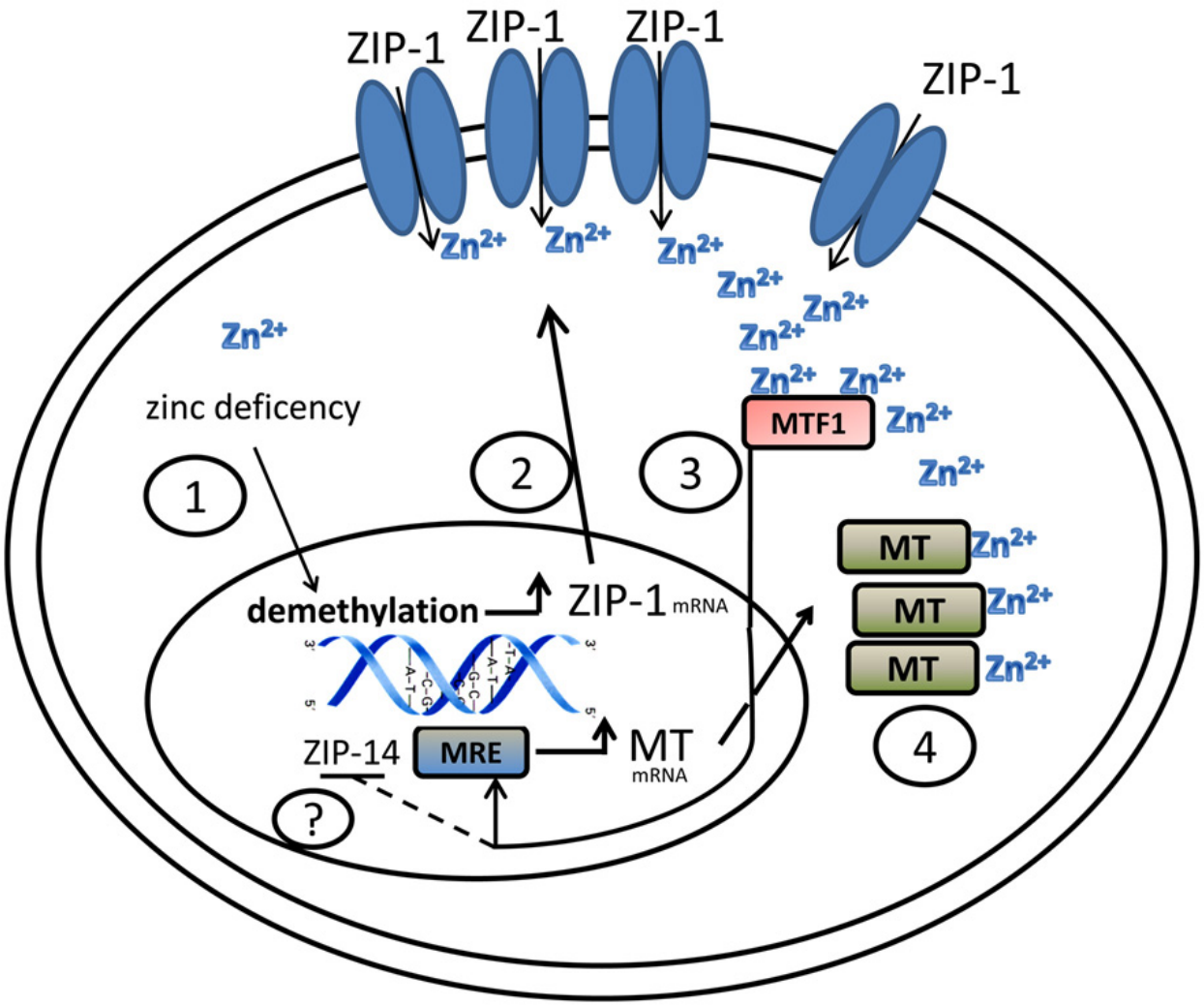

Fig. 1. Zinc deficiency (1) induces the mRNA expression of ZIP-1 and ZIP-1 protein accumulation on the cell membrane in myeloid cells (2). Increased ZIP-1 leads to a huge zinc influx into the cytoplasm which is detected by zinc-specific metal-response-binding transcription factor 1 (MTF1) (3). MTF1 migrates into the nucleus and specifically activates metal-responding elements (MRE) of the DNA. MTF1 then initiates the transcription of the zinc binding proteins such as MT, and may also block ZIP-14 gene expression. MTs bind elevated free zinc ions leading to a reconstituted zinc homeostasis (4).

Epigenetic changes turn genes on or off and specify which proteins are transcribed. DNA methylation is an epigenetic mechanism when methyl groups are incorporated into cytosine molecules by DNA methyltransferases (DNMTs), forming 5-methylcytosine. This process contributes to the silencing of genes. Epigenetic mechanisms are involved in the gene expression of zinc transporters and zinc-binding proteins. The aim of this study was to investigate whether

DNA methylation is involved in zinc homeostasis of myeloid cells. For this purpose, the myeloid cell line HL-60 was used. After incubation with the demethylating agent 5-Aza-2'-deoxycytidine (AZA) increased intracellular and total cellular zinc levels in HL-60 cells were observed after 24 h and 48 h. Real time PCR analyses of 14 ZIP and 9 ZnT zinc transporters showed significantly enhanced mRNA expression of the zinc importer ZIP-1 after AZA treatment over time. This could be substantiated in Western blot analyses in which ZIP-1 protein was also found to be enhanced after AZA treatment. Our data suggest that DNA methylation is at least one mechanism that regulates ZIP-1 gene expression, and that ZIP-1 is responsible for enhanced intracellular zinc levels. Interestingly, incubation with AZA resulted in increased mRNA accumulation of zinc binding proteins MT and S100A8/S100A9 after 48 h. MT mRNA was already enhanced after 24 h of AZA treatment. In addition, reduced expression of the zinc importer ZIP-14 and increased expression of the zinc exporter ZnT3 could also be observed. These results indicate a cellular counteracting effect to reduce excess zinc and restore zinc homeostasis. However, the observed mRNA enhancement might directly be linked to DNA-methylation, or because of activation of other genes. This might be the case for ZnT3 which is normally expressed in the brain, but not in leukocytes. From this data, it is conceivable that the activation/demethlyation of ZIP-1, e.g. triggered by low intracellular levels, leads to increased gene expression of ZIP1. The enhanced production of ZIP-1 protein and transfer to the plasma membrane results in a massive influx of zinc into the cytoplasm. This excess of zinc is recognized by zinc-specific metal-response-binding transcription factor 1 (MTF1). MTF1 binds to metal-responding elements (MRE) of the DNA in the nucleus. Within the nucleus MTF1 may promote the inhibition ZIP-14 gene expression and activates the transcription of the zinc binding proteins such as MT to compensate elevated zinc level (Fig. 1). In summary, these data indicate that DNA methylation is an important epigenetic mechanism affecting zinc binding proteins and zinc transporters, and, therefore, is involved in regulating zinc homeostasis in myeloid cells.

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