

## ABSTRACT

The role of sequence diversity in human metallothioneins (MTs) in cellular Zn<sup>2+</sup> homeostasis

The role of sequence diversity in human metallothioneins (MTs) in cellular Zn<sup>2+</sup> homeostasis remains not fully understood. To date, four human metallothionein isoforms have been identified (MT1, MT2, MT3, MT4), along with eight subisoforms of MT1 (MT1A, MT1B, MT1E, MT1F, MT1G, MT1H, MT1M(K), MT1X). Despite extensive research, comparative analysis of the biochemical properties of these isoforms is complicated by the variety of experimental methods used, and the focus on individual isoforms. Metallothioneins pose a challenge in research due to their lack of secondary structure and aromatic amino acids. What is more, Zn<sup>2+</sup>, with its weak spectral properties, is not the most frequently chosen metal for studying metal-metallothionein interactions.

The primary objective of this study was to produce and characterize all known human metallothionein isoforms and their natural variants (including MT1A T27N, MT1A K51R, MT1G A10del, MT1M T20K, MT4 C30Y, MT4 R31W, MT4 G48D) as well as the metalloprotein MT1HL1. Standardizing experimental techniques enabled reliable comparative analysis and improved the understanding of the role of sequence diversity in metallothioneins in Zn<sup>2+</sup> homeostasis.

The results obtained provided a better understanding of how sequence diversity in human metallothioneins affects their biophysical and biochemical properties under conditions of cellular Zn<sup>2+</sup> homeostasis. For the first time, all human metallothionein isoforms were produced at the protein level and compared using a standardized methodology. Furthermore, natural variants of metallothioneins and MT1HL1 were also produced and characterized for the first time. Affinities for Zn<sup>2+</sup> ions corresponding to all known binding sites in the studied metalloproteins were determined. The study also demonstrated how different isoforms and their metalated forms complement each other in terms of cellular labile Zn<sup>2+</sup> fluctuations, which is crucial for proper cellular physiology. Characterization of natural variants of human metallothionein isoforms highlighted how sequence diversity, resulting from single amino acid changes, affects the stability and reactivity of metallothioneins. Additionally, the high similarity of the biophysical properties of MT1HL1 compared to human metallothionein isoforms suggests that this metalloprotein should be considered a new MT isoform.

The results of this study represent a significant step towards understanding how sequence diversity in human metallothioneins influences Zn<sup>2+</sup> regulation and underscore the need for further research on protein-metal interactions and the development of advanced analytical techniques.