Evaluation of Manganese (Mn) Binding Proteins in Lung Carcinoma Cells

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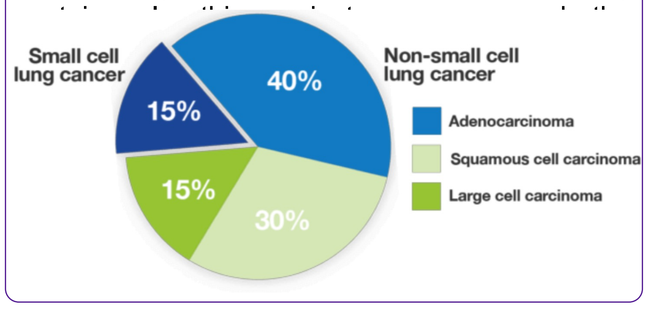
Introduction

Despite significant improvements in lung cancer therapies in recent years, lung cancer remains as the leading cause of cancer-related death due to its inclination to metastasize. Recent findings highlight the metastatic capacity of tumor cells as a result of overexpression of the Golgi integral membrane protein 4 (GOLIM4), which is frequently amplified in many human cancers, including lung squamous cell carcinoma (LUSC). GOLIM4 forms a complex with ATPase Secretory Pathway Ca^{2+} transporting 1 (ATP2C1) on the trans-Golgi and promotes pro-metastatic vesicle trafficking. Highly expressed GOLIM4 drives lung cancer growth and metastasis. Manganese (Mn) is an essential element that is present in tiny amounts in the human body. Mn treatment causes GOLIM4 degradation and inhibits the growth of chromosome 3q-amplified LUSC cells. To identify other factors that may mediate the antitumor functions of Mn, we performed a pull-down assay and identified several Mn binding



Hypothesis

We have shown that GOLIM4 is a Mn binding protein which is degraded upon Mn exposure. We found that Mn treatment effectively suppressed the growth of chromosome 3q-amplified lung cancer cells. We will examine the functions of these Mn binding proteins. We hypothesize that Mn functions by targeting several oncogenic proteins.

Results

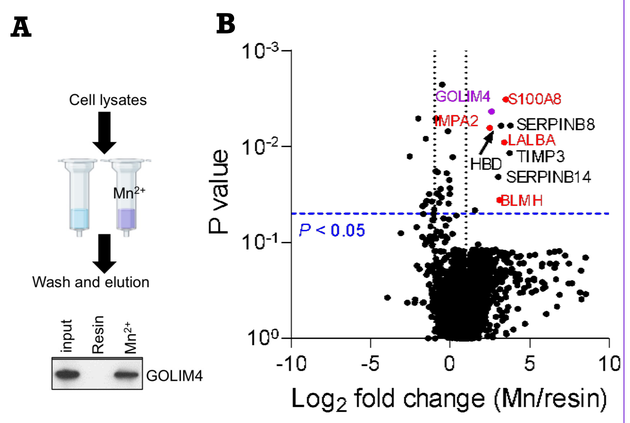


Fig. 1 **A.** Schematic flow of Mn pull-down assay. **B.** A volcano graph showing identified Mn binding proteins. Newly identified Mn binding proteins are indicated in red. We chose four proteins (BLMH, S100A8, SERPINB8, TIMP3) for further functional studies based on the peptide abundance.

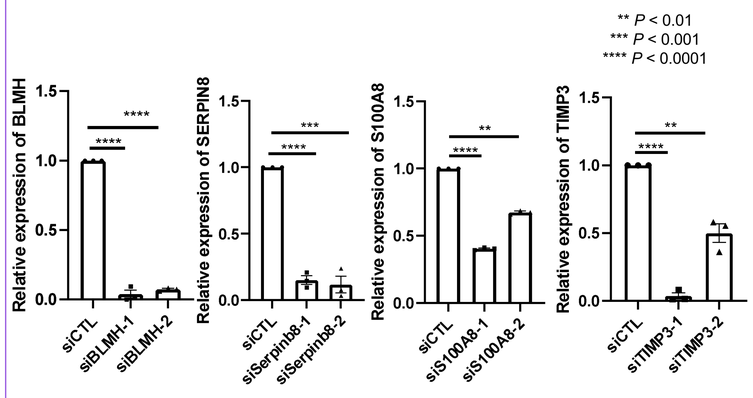


Fig. 2 H1299 cells were transfected with two different siRNAs against BLMH, S100A8, SERPINB8, TIMP3, or control siRNA (siCTL), and the mRNA levels of each gene were examined by quantitative real time PCR. Both siRNAs could effectively knock down the target genes.

Binding Proteins

BLMH

(Bleomycin Hydrolase) is a cytoplasmic peptidase involved in the inactivation of glycopeptide bleomycin.

SERPINB8

(Serpin Family B Member 8) is an enzyme produced by platelets and can bind to and inhibit the function of furin.

S100A8

(S100 Calcium Binding Protein A8) is a calcium binding protein involved in cell cycle progression and differentiation.

TIMP3

(TIMP Metallopeptidase Inhibitor 3) is a protein coding gene involved in degradation of the extracellular matrix (ECM).

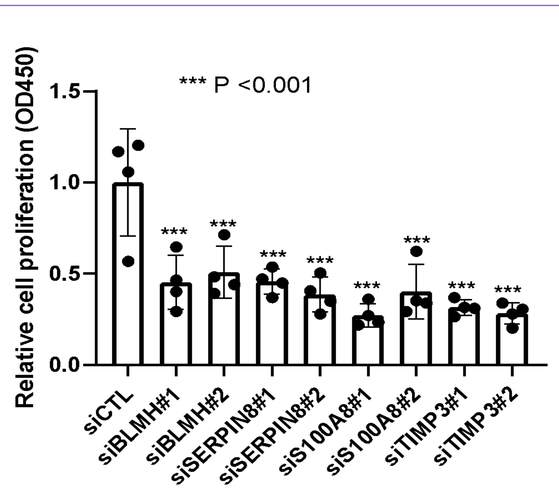


Fig. 3 H1299 cells were transfected with indicated siRNAs, and cell proliferation was determined by WST-1 assay. The results show that the two siRNAs exhibit similar activity in suppressing cancer cell proliferation. All these four genes are required for the proliferation of H1299 cancer cells.

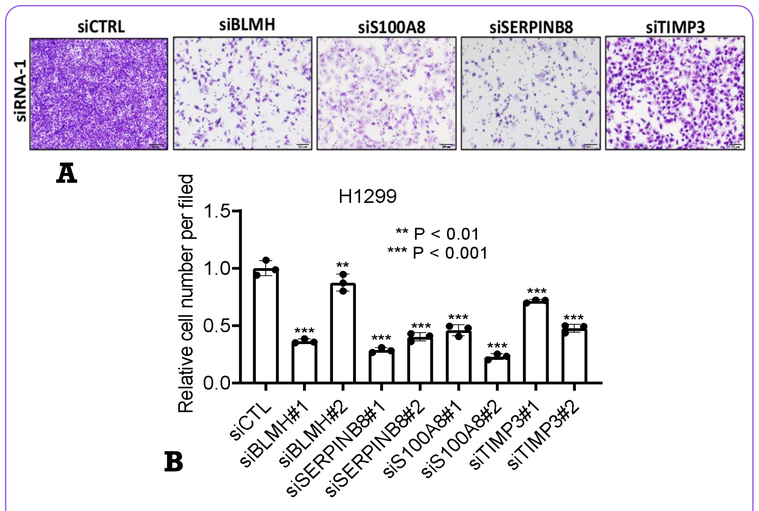


Fig. 4 H1299 cells were transfected with indicated siRNAs and cell migration was assessed using Boyden chamber transwell assay. The migrated cells were stained and counted. **A.** Images of migrated cells. **B.** Quantification of migrated cells. The results were normalized to siCTL. All these four proteins were required for the migration of H1299 cancer cells, although the effect of siTIMP3 is marginal.

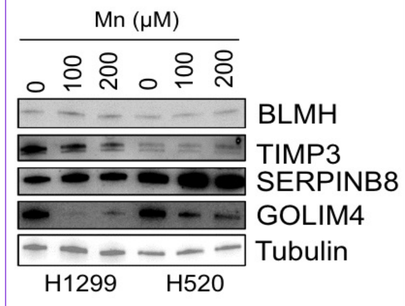


Fig. 5 H1299 and H520 cells were treated with (100 or 200 μM) or without Mn for 24 h, and the protein levels of BLMH, TIMP3, SERPINB8, and GOLIM4 proteins were determined by Western Blot. We found that Mn treatment significantly reduced the protein level of GOLIM4 but not the other three proteins, indicating that these proteins bind to Mn but are not degraded by Mn.

Methods

We identified several significant Mn binding proteins (BLMH, S100A8, SERPINB8, TIMP3) in a Mn pull-down assay. We used specific siRNAs to knock down these proteins and assess cell proliferation using the WST-1 method and cell migration using the Boyden chamber trans-well assay. In addition, we examined the levels of these proteins following Mn treatment by performing a Western blot to investigate if Mn caused degradation of these proteins.

Conclusion & Future Directions

We found that these Mn binding proteins play critical roles in cell proliferation and migration. However, Mn treatment did not result in degradation of these proteins, suggesting that they may not be primary Mn targets in chromosome 3q-amplified lung cancer cells. Thus, we conclude that GOLIM4, but not other Mn binding proteins, are responsible for Mn-induced cancer cell growth inhibition. In the future, we will focus on the functions of GOLIM4 in lung cancer growth and metastasis.

Acknowledgements

This project is supported by the 1R25CA240137-01A1 UPWARDS Training Program (Underrepresented Minorities Working Towards Research Diversity in Science) and CPRIT Research Training Award CPRIT Training Program (RP210028).

I would like to give a special thank you to my mentor, Dr. Xiaochao Tan, for educating and assisting me for the duration of this program.

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