The nuclear Zn transporter ZIP11 is necessary for the proliferation of HeLa cells

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Abstract

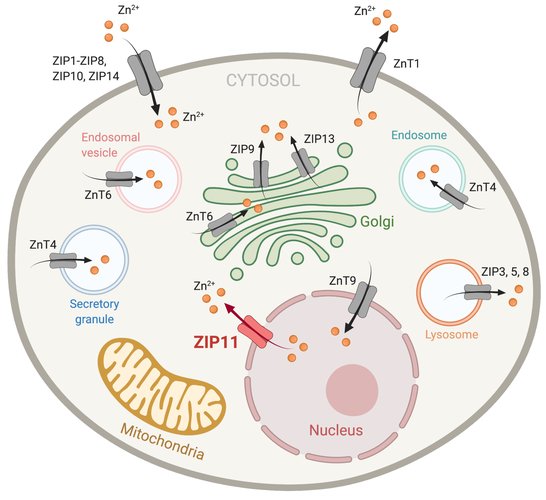
Zinc (Zn) is an essential trace element as part of several biological processes, including transcriptional regulation, signaling, and catalysis. A subcellular network of Zn transporters ensures the adequate distribution of Zn to maintain homeostasis. Among these, the family of importers Zrt/Irt-like protein (ZIP) constitutes 14 members (ZIP1-ZIP14) that mobilize Zn into the cytosol. Expression of these transporters varies among tissues and during developmental stages. The presence of ZIP transporters at various cellular locations is essential for defining the net cellular transport of Zn. Normally, the ion is bound to proteins or sequestered in organelles and vesicles.

Research has focused on Zn internalization in mammalian cells. However, little is known regarding Zn mobilization within the cells and the organelles, including the nucleus. ZIP11 is the only ZIP transporter localized in the nucleus of mammalian cells. However, the cellular role and the mechanism and direction of transport of ZIP11 are not defined. We hypothesized that ZIP11 is a nuclear Zn transporter essential to maintaining nuclear Zn homeostasis in mammalian cells. To test this idea, we knocked down *Zip11* in normal fibroblasts and HeLa cancer cells. Preliminary data shows that partial deletion of *Zip11* reduced the proliferation of HeLa cells, and when HeLa cells reached confluency, they acquired an epithelial morphology. None of these phenotypes were detected in HEK293T cells, suggesting that ZIP11 might be relevant for the proliferation of cancer cells. Our work has the potential to discover a novel molecular mechanism where nuclear Zn homeostasis is essential for cancer progression.

Introduction

- Zn is an abundant trace element for all organisms
- Zrt/Irt-like protein (ZIP) transporters mobilize Zn into the cytosol while ZnT's export Zn out of the cytosol into the extracellular milieu or vesicles and organelles
- **ZIP11** is proposed to present nuclear localization

Fig. 1: Schematic representation of zinc network in cells



Results

Fig. 2: *Zip11* shRNA-mediated KD impairs growth and alters cellular morphology

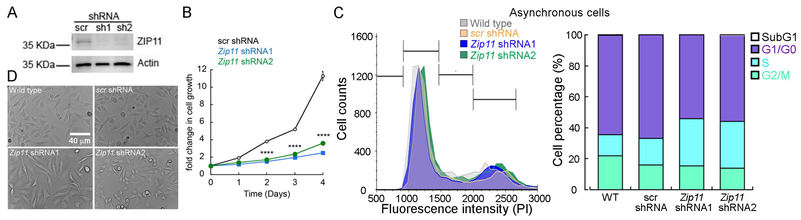


Fig. 3: Partial depletion of Zip11 decreased secretion and activity of extracellular matrix metalloproteases MMP2 and MMP9

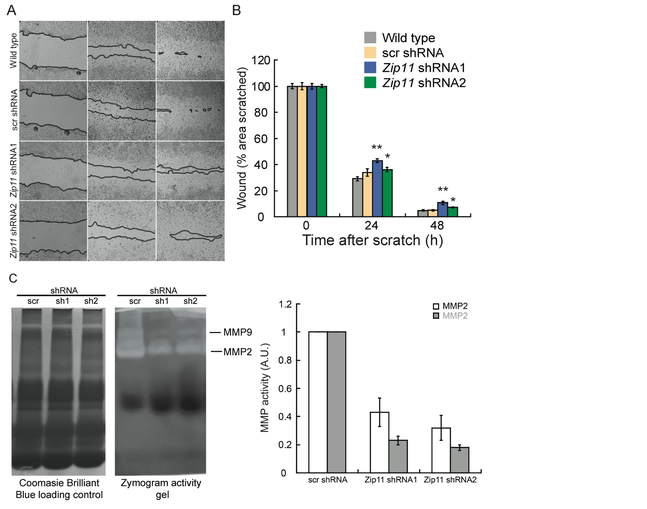


Fig. 4: Venn diagrams and volcano plots generated from RNA-Seq (DEseq2) analysis

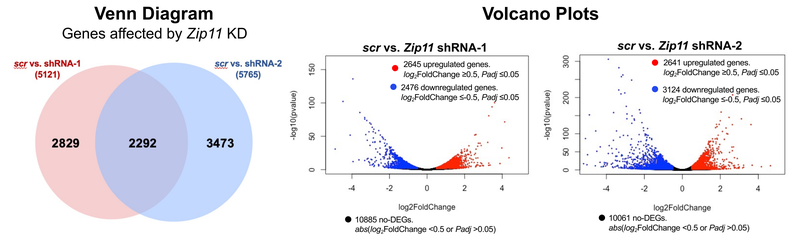


Fig. 5: Representative heat maps of genes involved in cell cycle, EMT and apoptosis. The data represents significant average changes in expression for sh1 and sh2 replicates. Upregulated genes (blue) and downregulated genes (red).

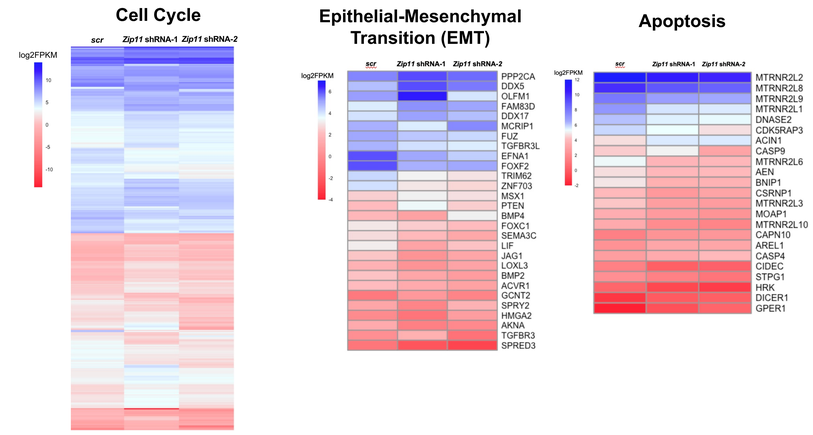
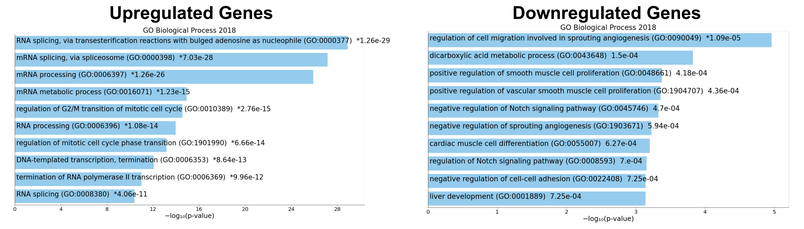


Fig. 6: Gene ontology shRNA1 analyses for upregulated and downregulated genes overlapping in *Zip11* and shRNA2, ranked by p-value



Conclusions

- Knockdown of *Zip11* in HeLa impaired proliferation
- MMP2 and MMP9 are significantly less active in *Zip11* KD cells
- RNA-Seq analyses showed dysregulation of cell cycle genes upon KD ZIP11

Future Directions

- Experiments on other cell lines to compare phenotypes to HeLa cells
- Validate the expression candidate genes found from heatmap data
- Cell cycle analysis of synchronized populations
- Investigate other pathways of interest for this phenotype (EMT, apoptosis)

Materials & Methods

Cell culture: Hela cells were cultured in DMEM 10% FBS in a humid atmosphere supplemented with 5% CO2 and with or without the indicated concentrations of ZnSO4.
Western blot: Protein was extracted in RIPA buffer and using an anti-Zip11 and anti-actin antibodies from Abclonal.
Zymographies: Concentrated supernants of each one of the transduced cell lines were run in 10% Native PAGE supplemented with bovine gelatin and incubated overnight in buffer containing $CaCl_2$.[1,2]
RNA-Seq: Libraries of ChIP-enriched DNA were prepared from 2 biological replicates following the Illumina strategy (Illumina, San Diego, CA, USA).
Bioinformatic analyses: The Zip11 RNA-Seq dataset from Hela cells transduced with scr shRNA and two different shRNAs against Zip11 was performed by BGI and independent replicates were comparatively analyzed using Excel, R Studio, and Enrichr.

Acknowledgements

- Padilla-Benavides lab members
- Wesleyan MB&B Department

References

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