Lentivirus-mediated Knockdown of ERK3 Inhibits Migration of Gastric Cancer Cells

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Introduction

- Stomach cancer is the 5th most common cancer
- Stomach cancer often metastasizes, making it the 3rd leading cause of cancer-related deaths
- Clinical data has shown that ERK3 may play an inhibitory role in gastric cancer metastasis

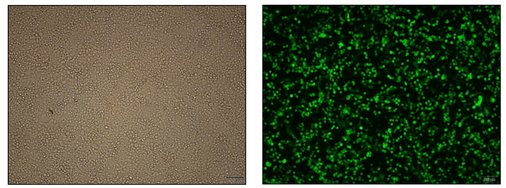
Objective

To determine the role of ERK3 in vitro by knocking down its expression using short-hairpin RNAs (shRNAs).

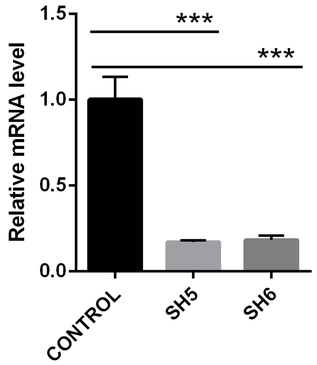
Methods

- Design two shRNA targets
- Lentivirus plasmid construction
- Lentivirus packaging
- Selection of stable knockdown cell line
- Confirm knockdown efficiency by quantitative PCR (qPCR)
- Cell migration assay
- Cell proliferation assays
  - Colony formation
  - MTT

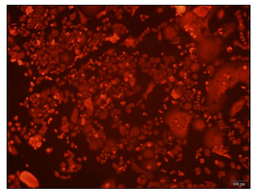
Results



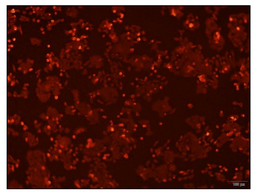
In the selection of a stable knockdown cell line, green fluorescence indicates successful infection by lentivirus.



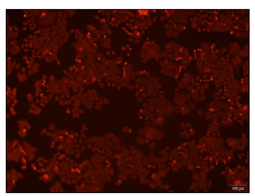
Following lentivirus infection, ERK3 mRNA is significantly downregulated.



CONTROL

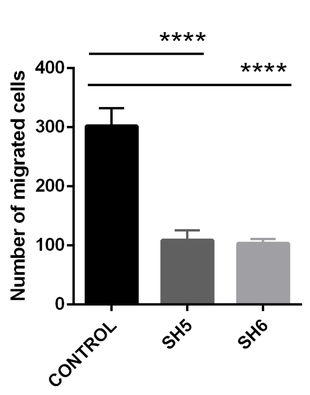


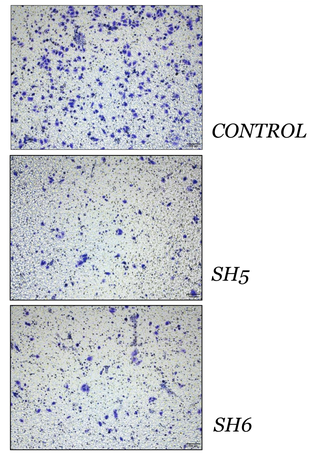
SH5



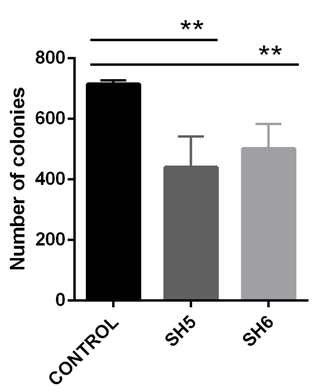
SH6

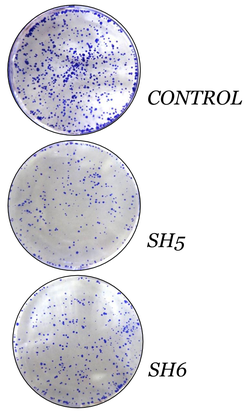
Immunofluorescent staining indicates less ERK3 present (red) in the knockdown cell lines.



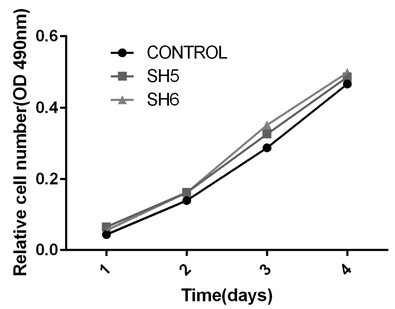


Knockdown of ERK3 inhibits cancer cell migration.





Knockdown of ERK3 inhibits colony formation.



Knockdown of ERK3 has no effect on the proliferation of cancer cells in the MTT assay.

Discussion

- These experiments have demonstrated the effect of ERK3 on cells' migratory and proliferative abilities in vitro
- An inconsistency arose in the results from the colony-formation and MTT assay; in vivo experiments could clarify the proliferative capacity
- In vivo experiments to test the tumorigenesis and metastatic potential of this cell line are needed to confirm the function of ERK3

Questions

- What contributed to the differing results of the colony-formation assay and MTT assay?
- Why does this experimental data, unlike clinical data, suggest that ERK3 contributes to metastasis?

Conclusion

ERK3 contributes to the metastasis and growth of gastric cancer cells in vitro.

Acknowledgements

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