LIVE1 plays a role in maintenance of glioblastoma vascular integrity

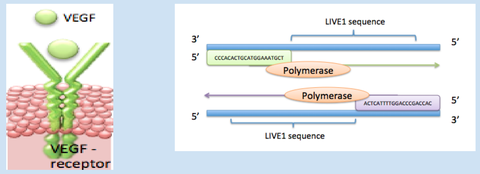
Oleksandra Kaskun[1], Rohit Sachdeva[1], Sandra Smilijanic[1,2], Jenny Wang[1], Megan Wu[1], Sunit Das[1, 2]

1.Brain Tumour Research Centre, SickKids, Toronto, ON, Canada

2.St. Michael's Hospital, Toronto, ON, Canada

Background: LIVE1

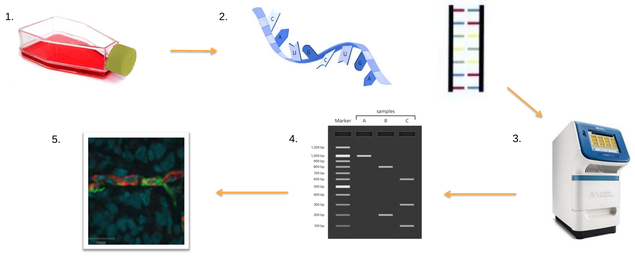
Glioblastoma Multiform (GBM) is a highly malignant brain tumour with high vasculature, and while aggressive treatment results in immediate disease control, relapse is invariable. The lab has identified a VEGF-A-responsive lincRNA near the *VEGFR1* gene, which we termed lincRNA-VEGFR1 (LIVE1). LIVE1 was found to exert transcriptional control over the *VEGFR1* gene and direct angiogenesis *in vitro*.



Objective

To determine endothelium/pericyte differentiation along the vascular cell lineage within glioma stem cell (GSC) lines that greatly express LIVE1.

Materials and Methods



1. Glioma stem cells (HFNS to normalize) were cultured as spheres in stem cell culture media with growth factors at 37ºC.
2. Total RNA was extracted using RNeasy Micro Kit and transcribed into cDNA with High-Capacity cDNA Reverse Transcription Kit and amplified with PCR.
3. qPCR using 18S as housekeeping gene and SYBR Green.
4. PCR and gel electrophoresis on 2.5% agrose gel.
5. Immunofluorescence staining (IF) on tissue sections of xenografts.

Greatest LIVE1 Expression

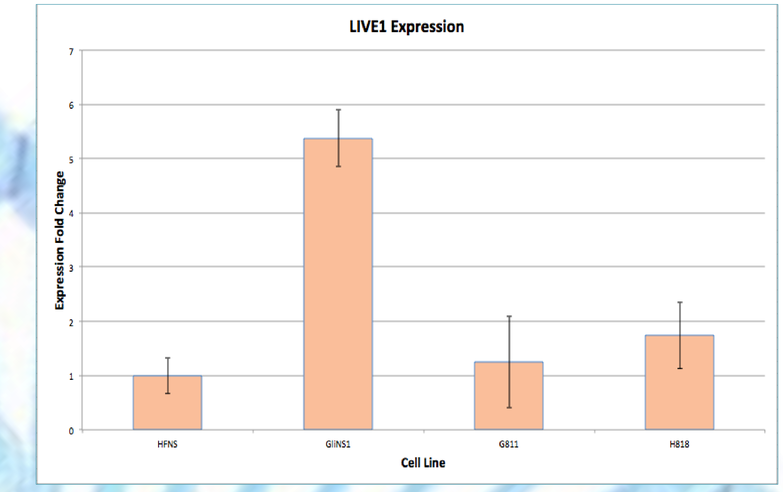


Figure 1: qPCR analysis shown LIVE1 expression fold change normalized to HFNS. Error bars represent standard deviation. GliNS1 cell lines have the greatest LIVE1 expression compared to other GSC lines.

LIVE1 in GSC

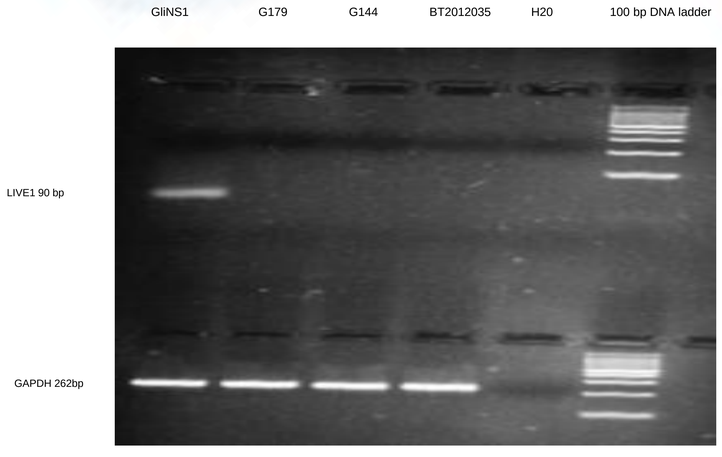
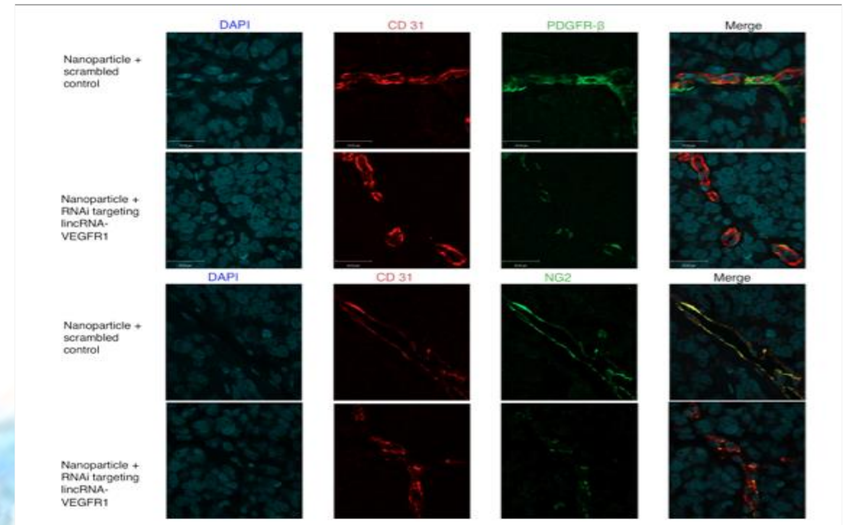


Figure 2: Gel electrophoresis post PCR confirmed GliNS1 is a LIVE1 high-expressor. GAPDH was used as housekeeping gene.

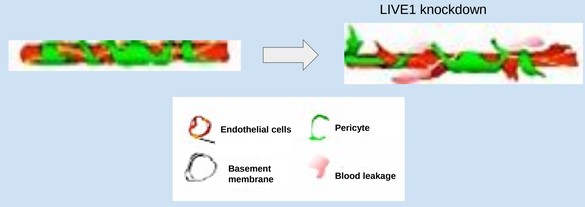
Endocyte/ Pericyte Cell Fate

Figure 3: Immunofluorescence staining of LIVE1 high-expressor GliNS1 xenograft tissue sections with DAPI, a nucleus marker, CD 31, an endothelium marker, and PDGFR-β and NG2, pericyte markers. In the LIVE1 knockdown model, both PDGFR-β and NG2 show decreased pericyte expression and reduce the percentage of pericyte coverage.



Conclusion

Our results show a lower percentage of pericyte coverage in LIVE1 knockdown mouse model, indicating LIVE1 plays a role in endothelium-pericyte signaling networks contributing to tumor angiogenesis and metastasis.



Acknowledgements

I would like to thank the Brain Tumour Foundation of Canada Brain Tumour Research Studentship generously funded by gifts from Brain Tumour Foundation of Canada Donors, as well as the Labatt Brain Tumour Research Centre and the Das lab for the opportunity to conduct this research and their continuous support.