A Novel VEGF-Responsive lincRNA Regulates Angiogenesis in Glioblastoma

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Introduction

Large intergenic RNAs (lincRNA) are involved in numerous cellular processes, including those relevant to normal development and cancer progression. We speculated that differential lincRNAs expression would be important to fate specification in endothelial cells and in angiogenesis in glioblastoma.

Methods

A custom microarray was used to profile long non-coding transcripts in human vascular endothelium in two-dimensional versus three-dimensional pro-angiogenic cultures, with or without VEGF-A165.

Results

Using a linc array in HUVECs pushed toward endothelial maturation through treatment with VEGF, we identified a VEGF-A-responsive lincRNA near the VEGFR1 gene, which we termed LiVE1 (lincRNA-VEGFR1). Through knockdown and over-expression studies, we found LiVE1 to exert transcriptional control over multiple genes involved in the angiogenesis signaling cascade, including VEGFR1, VEGFR2, and Flt1, and to direct vascular maturation in vitro. Given its significance to endothelial cell differentiation and maturation, we postulated that LiVE1 could have a role in neoplastic angiogenesis in glioblastoma. Indeed, we found that LiVE1 is highly expressed in glioblastoma and is enriched in the CD133+ glioma stem cell and CD133+CD144+ endothelial progenitor populations. In vivo knockdown of the LIVE1 using nanoparticles-based RNAi decreased microvascular density and tumour volume in a heterotopic glioblastoma xenograft model, and resulted in local tumor control in an orthotopic xenograft model.

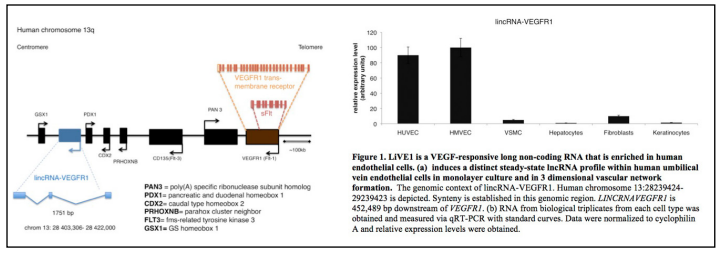

Figure 1. LiVE1 is a VEGF-responsive long non-coding RNA that is enriched in human endothelial cells. (a) induces a distinct steady-state lncRNA profile within human umbilical vein endothelial cells in monolayer culture and in 3 dimensional vascular network formation. The genomic context of lincRNA-VEGFR1. Human chromosome 13:28239424-29239423 is depicted. Synteny is established in this genomic region. *LINCRNAVEGFR1* is 452,489 bp downstream of *VEGFR1*. (b) RNA from biological triplicates from each cell type was obtained and measured via qRT-PCR with standard curves. Data were normalized to cyclophilin A and relative expression levels were obtained.

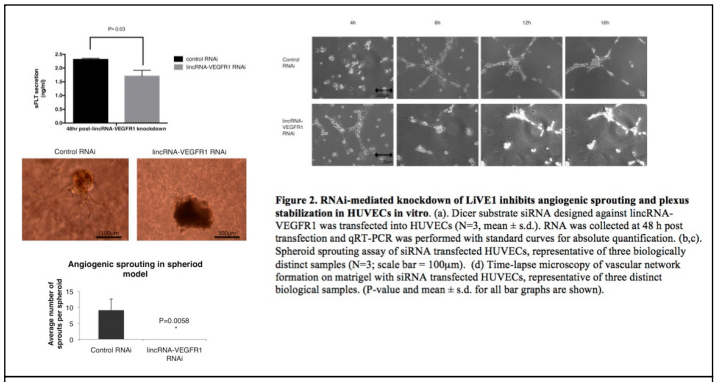

Figure 2. RNAi-mediated knockdown of LiVE1 inhibits angiogenic sprouting and plexus stabilization in HUVECs in vitro. (a). Dicer substrate siRNA designed against lincRNA-VEGFR1 was transfected into HUVECs (N=3, mean ± s.d.). RNA was collected at 48 h post transfection and qRT-PCR was performed with standard curves for absolute quantification. (b,c). Spheroid sprouting assay of siRNA transfected HUVECs, representative of three biologically distinct samples (N=3; scale bar = 100μm). (d) Time-lapse microscopy of vascular network formation on matrigel with siRNA transfected HUVECs, representative of three distinct biological samples. (P-value and mean ± s.d. for all bar graphs are shown).

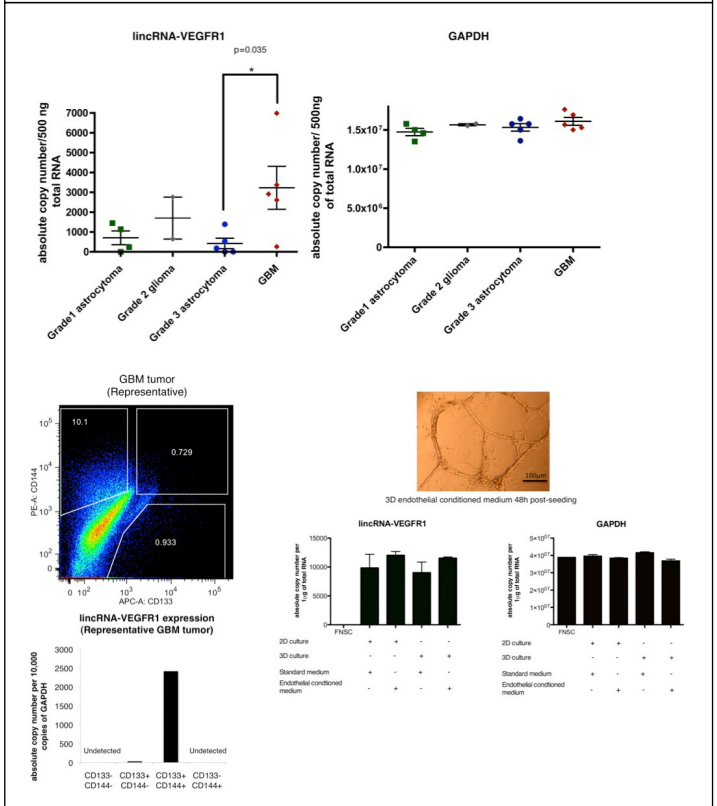

Figure 3. LiVE1 expression increases with glioma grade, and is enriched in CD133$^+$CD144$^-$ GSCs and CD133$^+$CD144$^+$ neoplastic endothelial progenitor cells. (a) RNA was isolated from 16 gliomas (4 Grade I, 2 Grade II, 6 Grade III, and 7 Grade IV). Absolute copy number was determined with standard curve and all absolute quantification was normalized to % RNA recovery and first strand efficiency. (b) Expression of lincRNA-VEGFR1 across the four intra-tumoural cell fractions, expressed in absolute copy number relative to 10,000 copies of GAPDH transcript (representative figure; N=3). (c) G144 GSCs were grown in either standard stem cell medium or endothelial-conditioned medium and seeded on 3D Matrigel (N=4; scale bar = 100μm). (d) LincRNA-VEGFR1 expression was determined by absolute quantification with qRT-PCR, all values were normalized to exogenous luciferase RNA for RNA recovery and first strand efficiency. Fetal neural stem cells (FNS) were used as a control. (N=4).

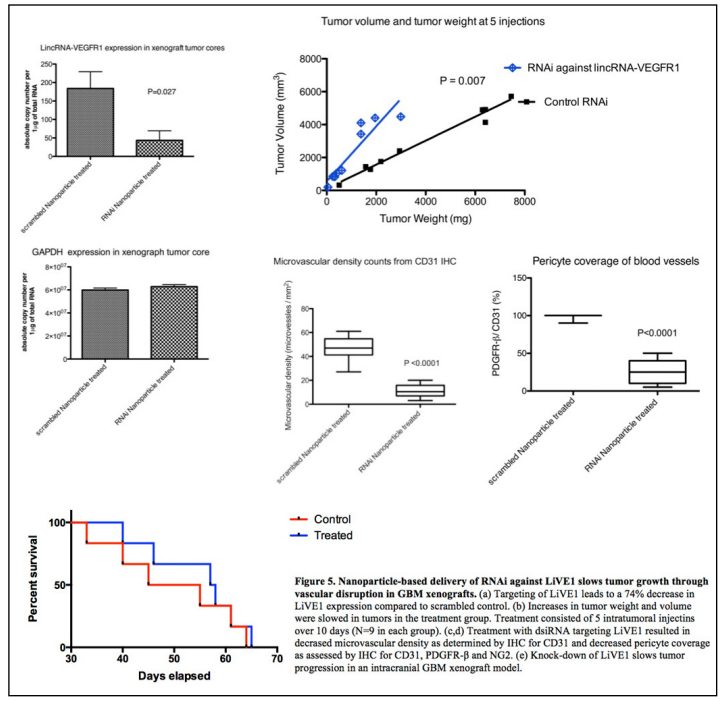

Figure 5. Nanoparticle-based delivery of RNAi against LiVE1 slows tumor growth through vascular disruption in GBM xenografts. (a) Targeting of LiVE1 leads to a 74% decrease in LiVE1 expression compared to scrambled control. (b) Increases in tumor weight and volume were slowed in tumors in the treatment group. Treatment consisted of 5 intratumoral injectins over 10 days (N=9 in each group). (c,d) Treatment with dsiRNA targeting LiVE1 resulted in decrased microvascular density as determined by IHC for CD31 and decreased pericyte coverage as assessed by IHC for CD31, PDGFR-β and NG2. (e) Knock-down of LiVE1 slows tumor progression in an intracranial GBM xenograft model.

Conclusions

Our studies delineate a linc-dependent molecular mechanism for angiogenesis in glioblastoma hat has significant therapeutic potential and merit attention as targets for pharmaceutical innovation. Knockdown of LiVE1 may be an effective means to target vascular neogenesis in glioblastoma.

Learning Objectives

By the conclusion of this session, participants should 1) Understand the identity of long non-coding RNAs and their relevance to cell biology; 2) Understand the role of LiVE1 in vascular maturation in glioblastoma.

References

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