The neuroblastoma-specific lincRNA NESPR regulates noradrenergic cell identity

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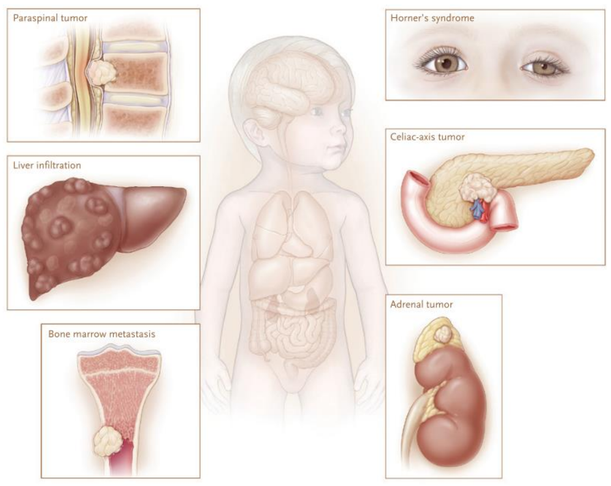
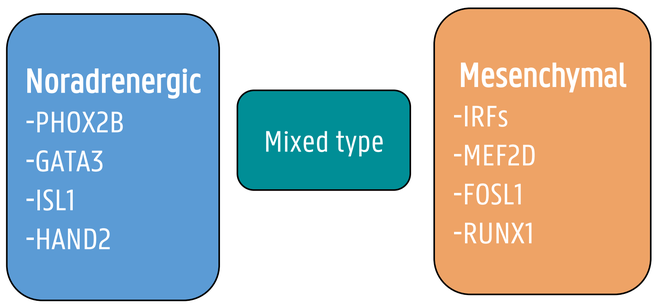
INTRODUCTION : NEUROBLASTOMA, A PEDIATRIC CANCER WITH 3 SUBTYPES

A.



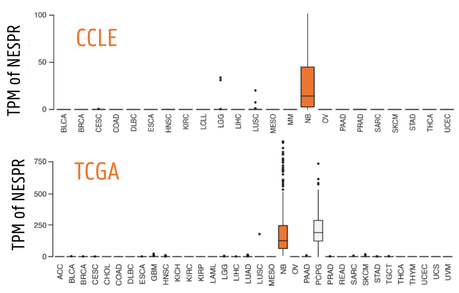
B.



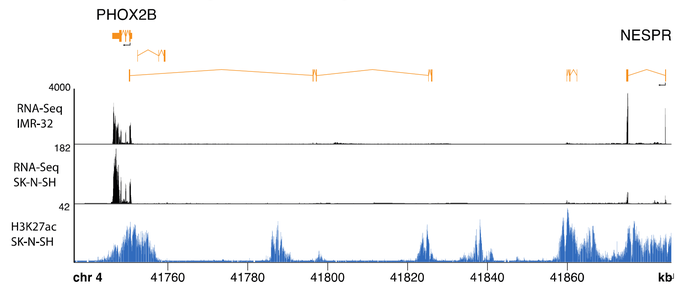
A. Neuroblastoma is a childhood cancer of the sympathetic nervous system that is diagnosed at a median age of 17 months. A majority of tumors arise from adrenal medulla with and typically metastasize to regional lymph nodes and to the bone marrow by means of the hematopoietic system. B. There are three types of identity in neuroblastoma cell lines: a sympathetic noradrenergic identity, defined by a core regulatory circuitry (CRC) module including the PHOX2B, HAND2, ISL1 and GATA3 transcription factors (TFs); a "mixed" type and NCC-like identity, driven by a CRC module containing IRFs and FOS TFs.

RESULT #1: NESPR IS NEUROBLASTOMA-SPECIFIC & TRANSCRIBED FROM PHOX2B SUPER-ENHANCER

A. NESPR expression across tumors

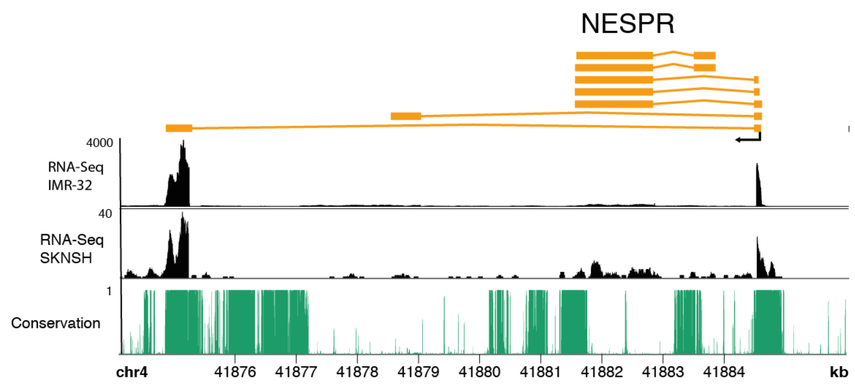


B. NESPR genomic neighbourhood



A. Pan-cancer lncRNA expression analysis comparing over 30 different tumor types resulted in the identification of the neuroblastoma-specific lncRNA NESPR (NEuroblastoma Specific Phox2B Regulatory RNA). B. RNA sequencing data and H3K27ac ChIP-Seq data indicated that NESPR is transcribed fromthe the PHOX2B super-enhancer.

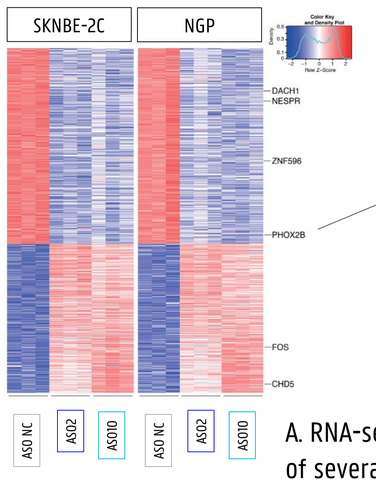
RESULT #2: NESPR IS EFFICIENTLY SPLICED AND HIGHLY CONSERVED IN MAMMALS

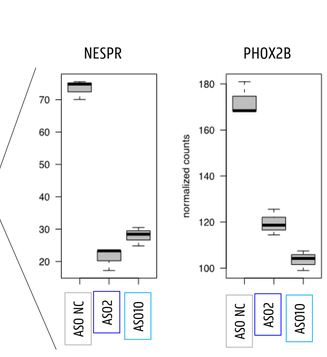


NESPR is abundantly expressed, efficiently spliced and highly conserved in mammals

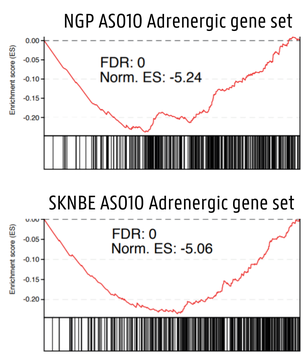
RESULT #3: NESPR PARTICIPATES IN NORADRENERGIC IDENTITY REGULATION

A. NESPR knock-down in NB cell lines



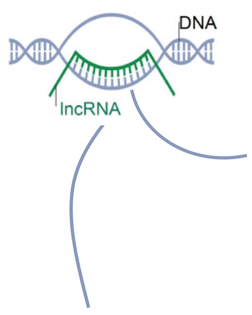


B. Gene Set Enrichement Analysis

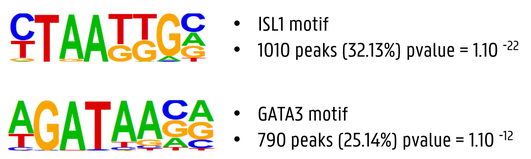


A. RNA-sequencing of ASO-treated neuroblastoma cell lines revealed a significant reduction of several neuroblastoma master regulators including PHOX2B. B. Adrenergic gene set was enriched in genes deregulated by ASO treatment of NESPR in neuroblastoma cell lines.

RESULT #4: NESPR BINDING SITES OVERLAP WITH NORADRENERGIC CRC TFS

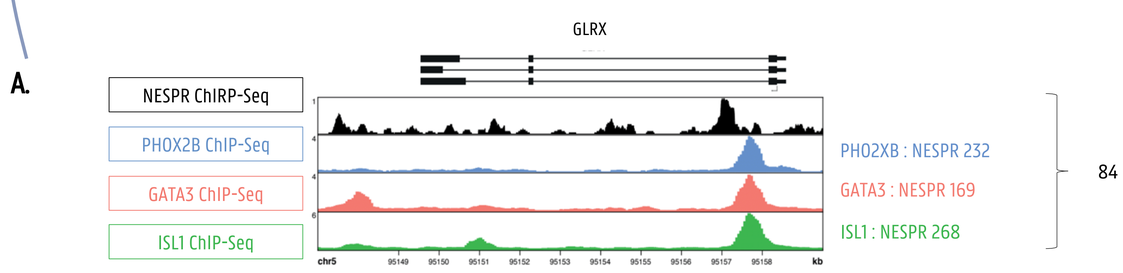


B. HOMER Motif analysis in NESPR-captured DNA



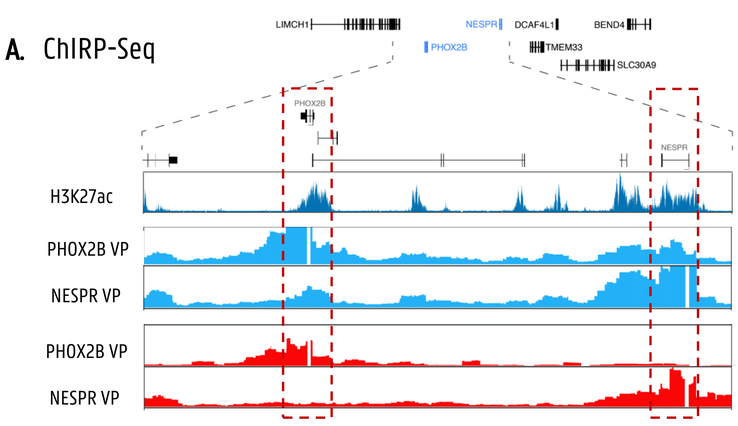
A. ChIRP-Seq revealed that DNA regions bound by NESPR overlap with that of noradrenergic TFs.
B. DNA fragments bound by NESPR was enriched in ISL1 and GATA3 TF motif.

A.

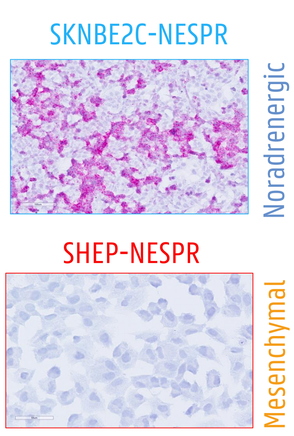


RESULT #5: PHOX2B & NESPR REGION IN NORADRENERGIC VS. MESENCHYMAL CELLS

A. ChIRP-Seq



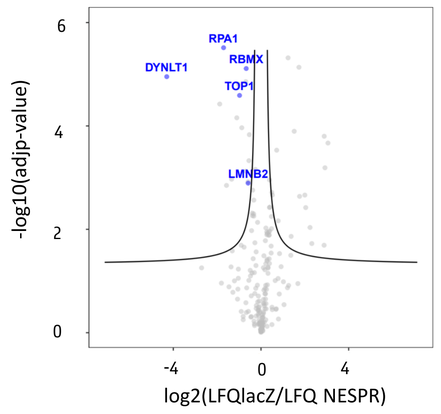
B. *In situ* hybridization



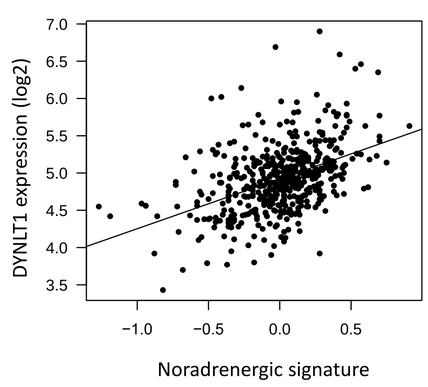
A. Using 4C-sequencing we could demonstrate a long-range interaction between the NESPR locus and the PHOX2B promoter (blue), suggesting that NESPR may act as a regulator of PHOX2B expression. This interaction was not observed in mesenchymal cells (red). B. Only the noradrenergic cell line SKNBE-2c showed positive staining for NESPR.

RESULT #6: NESPR ASSOCIATED PROTEIN ARE CORRELATED TO NORADRENERGIC GS

A. NESPR protein interactors



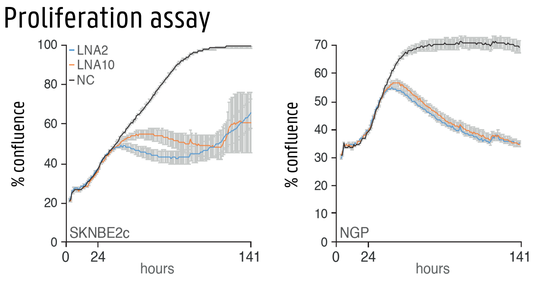
B. DYNLT1 associates to noradrenergic signature



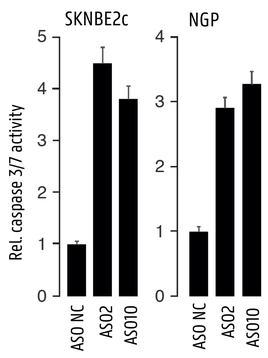
A. NESPR pull-down followed by mass-spectrometry (ChIRP-MS) revealed several potential protein interactors of NESPR, suggesting NESPR may recruit these factors to regulate PHOX2B expression. B. Expression of potential interactors is correlated to noradrenergic gene signature.

RESULT #7: NESPR KNOCK-DOWN IMPAIRS NEUROBLASTOMA CELL PROLIFERATION

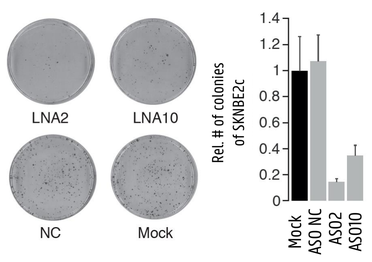
A. Proliferation assay



B. Apoptosis

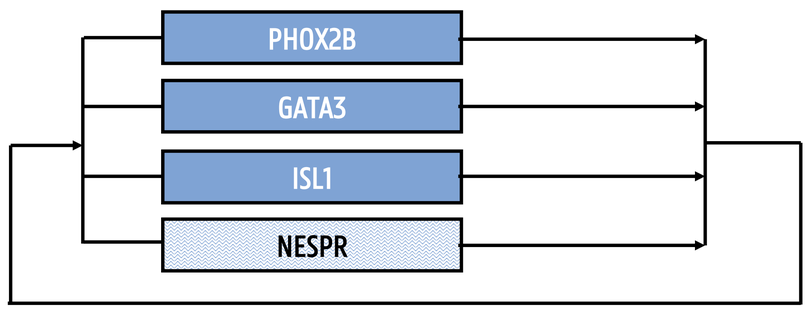


C. Colony formation assay



A. Knockdown of NESPR resulted in a significant decrease in cell growth, as evidenced by cell viability assays and real-time cell monitoring. B. Anti-growth effects of NESPR KDs were accompanied by an increase in apoptosis. C. NESPR ASO-treated cells also showed an impaired ability to form colonies.

CONCLUSION : THE NORADRENERGIC CORE REGULATORY CIRCUITRY



Overall our data suggest that the circuit of TF which regulate noradrenergic identity in neuroblastoma involve the lincRNA NESPR. These results underline the importance of lncRNAs in neuroblastoma and have prompted us to investigate NESPR's therapeutic potential.