HXR9 Inhibits the HOX-PBX Cluster, Inducing Glioma Apoptosis and Cell Cycle Arrest

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Introduction

- Primary brain tumors rank as the #1 cancer in terms of years of life lost
- Gliomas represent the majority (80%) of brain tumors and ATRX-deficient gliomas, i.e., astrocytoma, have the worst prognosis - average six years of survival.
- *ATRX*-loss gliomas cooccur with *IDH*-mut, *Tp53*-mut, and 1p/19q non-co-deletion.
- Current treatments include surgery, radiation and chemotherapy with significant side effects, including high morbidity and mortality.
- ATRX is a globally repressive chromatin remodeler through complexing with H3.3 and DAXX, depositing histone groups on chromatin.
- *HOX* genes are upregulated in developing fetuses to promote cell proliferation for development of the hindbrain and somites
- *HOX* is overexpressed in ATRX deficient gliomas.
- Research into the mechanism of ATRX deficiencies in these tumors will have great implications for survival.
- HXR9 is small peptide sequence that inhibits heterodimerization of HOX:PBX, inhibiting cell proliferation
- HXR9 is potent, clinically effective and blood-brain-barrier permeable peptide
- **Our aim is to establish HXR9 validity in promoting glioma stem cells apoptosis and elucidate the mechanism of action to better understand the role of HOX in gliomagenesis**

Methods

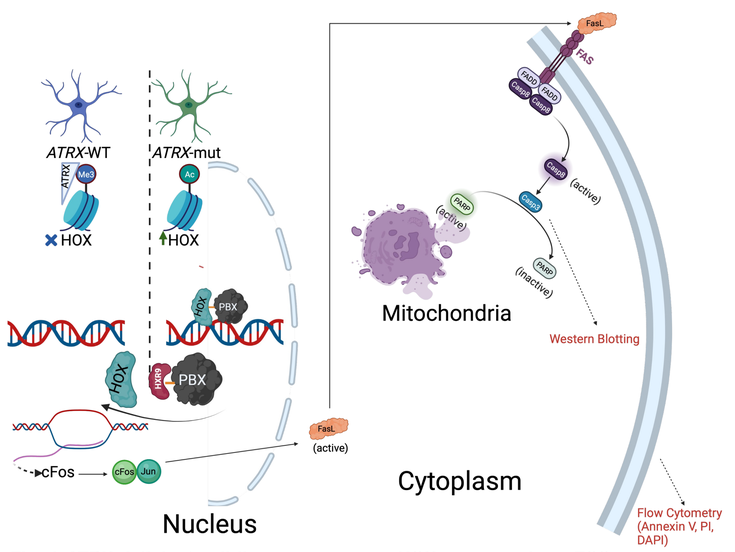


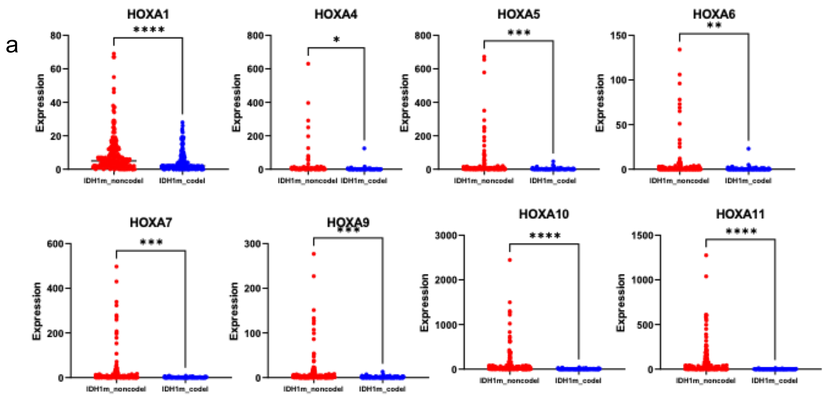
Fig. 1 ATRX-deficient cell lines overexpress HOX, suppressing *cFOS*, an apoptosis promoting factor. By competitively inhibiting the HOX-PBX complex, HXR9 may restore caspase-mediated apoptosis. (Made with *Biorender.com*)

Patient-Derived Glioma Stem Cell Lines	Characteristics
GS5-22	*IDH*-mut, *Tp53*-mut, *ATRX*-mut
TS603	*IDH*-mut, *Tp53*-mut, *ATRX*-WT

Table 1 Patient-derived glioma stem cells (GSCs) treated were the GS5-22 and TS603 cell lines.

Results

RNA-seq analysis shows increased expression of HOXA cluster genes in ATRX deficient gliomas



High expression of HOXA cluster genes is associated with poor prognosis in ATRX deficient gliomas

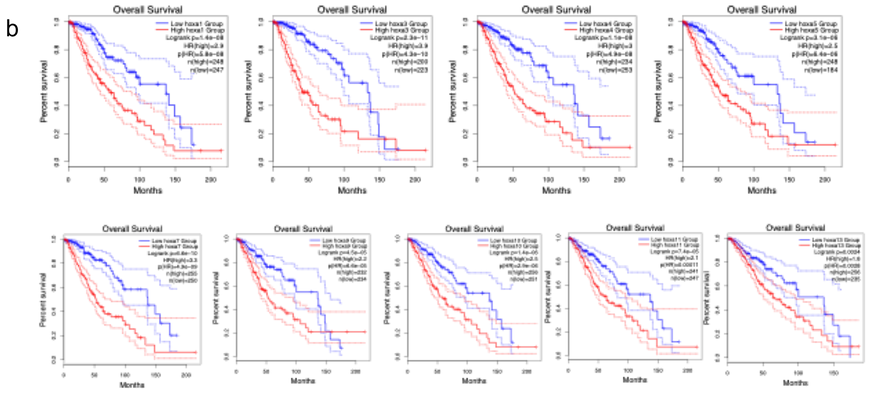


Fig. 2 HOX is overexpressed in ATRX-mut, IDH1-mut, 1p/19q non-co-deleted backgrounds (a) and correlates with lower overall survival (b). * <0.5, ** <0.01, *<0.001, ****<0.0001**

Dose dependent escalation of HXR9 drives patient derived gliomas towards apoptosis

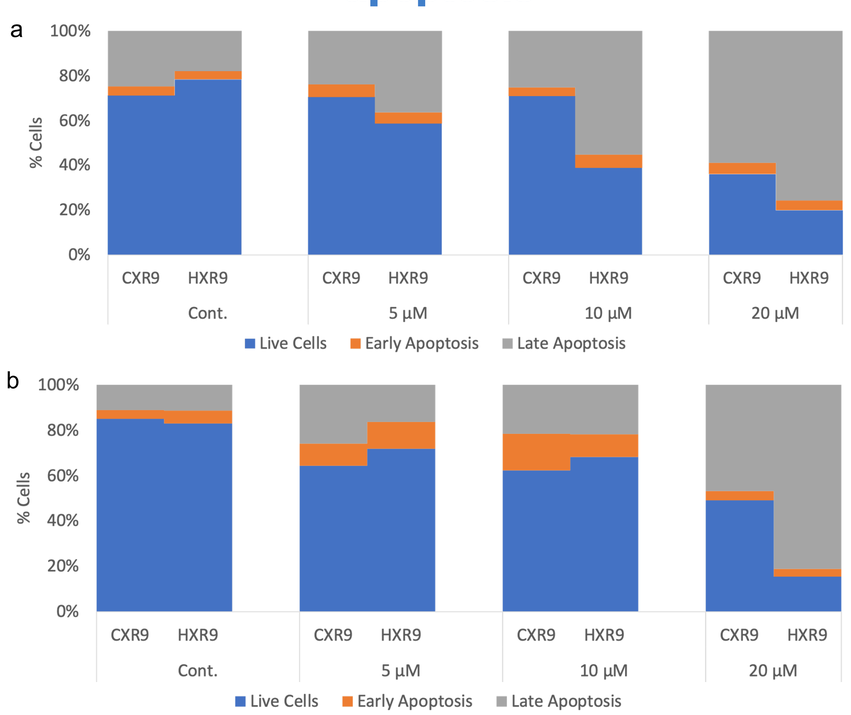


Fig. 3 HXR9 increases late apoptosis by 17% (5 µM) and 30% (20 µM) in GS5-22 *ATRX*-mut (a) and 34% (20 µM) with 2% increase in early apoptosis (5 µM) in TS603 *ATRX*-wt (b) glioma stem cells

Decrease in S-Phase of cell cycle upon treatment with HRX9 in patient derived gliomas

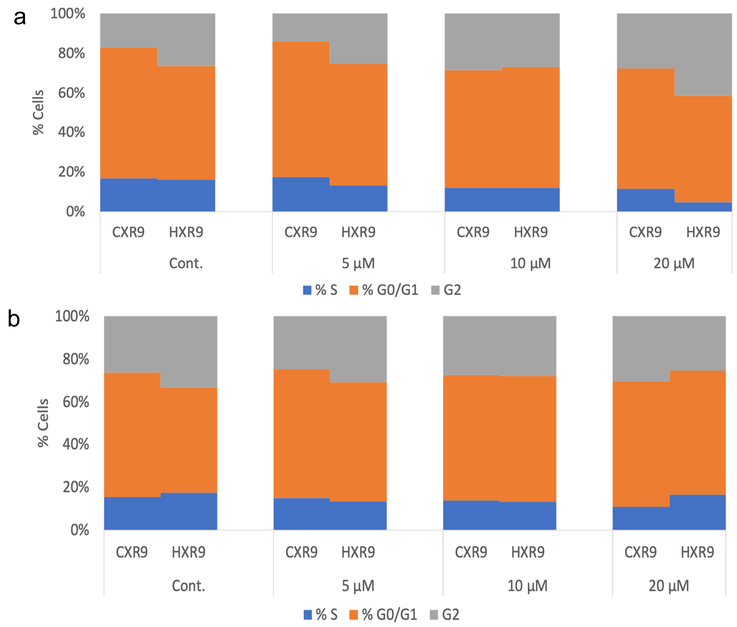


Fig. 4 HXR9 induces cell cycle arrest by reducing the % of GS5-22 cells in the synthetic (S)-phase by 4% and 5% (a) and the % of TS603 cells in both the S and growth 2 (G2) phases by 1.5% and 6% (b), at 5 µM and 20 µM, respectively

Conclusions

- Inhibition of HOXA cluster genes using HRX9 peptide, suggest its translational role in preclinical setting.
- Increase in apoptosis and reduced proliferation in HXR9 treated gliomas, open opportunity for in-vivo experiments.

Future Directions

- I plan to do qPCR to check if the HOX is suppressed.
- I can do western of a wide variety of proteins to confirm apoptosis.
- I can check the global transcriptional profile through RNA sequencing to detect different pathways the HXR9 peptide modulates.
- I can test HXR9 in TMZ-resistant, CD133+ stem cell lines.

Acknowledgements

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References

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