Targeting the Hippo Signaling Pathway in Atypical Teratoid Rhabdoid Tumor

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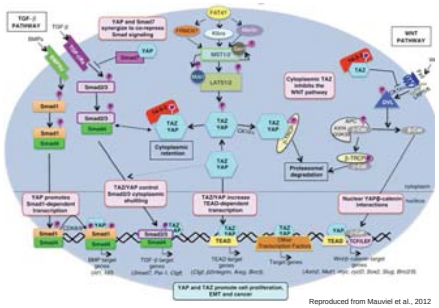
Introduction

•Atypical Teratoid Rhabdoid Tumor (ATRT) is a rare, highly malignant pediatric central nervous system tumor. The prognosis is poor, with a 2-year survival rate estimated at 15%.[1]

•The Hippo signaling pathway, a key mechanism for organ maintenance and growth, has been shown to be dysregulated in numerous cancers including medulloblastoma, glioblastoma, meningioma, and retinoblastoma. [2,3,4,5]

• Most Hippo abnormalities center around the common endpoint in the pathway – a set of transcriptional co-activators YAP and TAZ. An initial RNA sequencing screen showed Hippo dysregulation in ATRT. [6]



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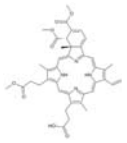
•A set of kinases regulate YAP/TAZ activity, which complex with transcription factors that are responsible for cell growth and differentiation. [6]

•With suspected Hippo dysfunction in ATRT, we hypothesized that YAP/TAZ could be a viable therapeutic target in the treatment of ATRT.

•Verteporfin, a YAP/TEAD1 small molecule inhibitor, has been shown to suppress cell proliferation in retinoblastoma. [5]

•Hippo involvement in ATRT was evaluated using RT-qPCR and Western blotting. Verteporfin inhibitory efficacy was then investigated using dose-response assays.



Molecular Structure of Verteporfin

Objectives

1. Determine whether Hippo activity is aberrant in ATRT
2. Determine the efficacy of Verteporfin therapy in atypical teratoid rhabdoid tumor.
3. Determine whether Verteporfin warrants further study in atypical teratoid rhabdoid tumor.

Results

Hippo Gene Expression Profile in ATRT

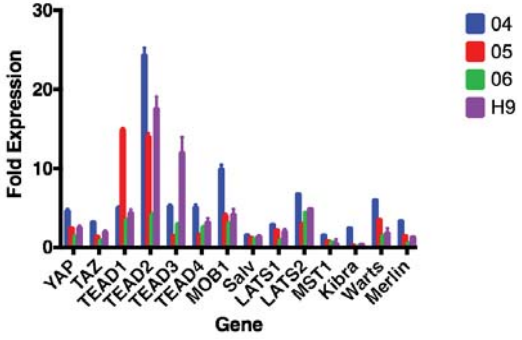


Figure 1. mRNA expression profile of ATRT cells (CHLA-04-ATRT, CHLA-05-ATRT, CHLA-06-ATRT) and neural stem cells (H9). RNA was extracted and qRT-PCR was performed. The neural stem cell line H9 was used as a positive control and all data was normalized to GAPDH. Gene expression is represented using fold-change compared to a pediatric non-tumor pooled brain tissue by the negative delta CT method

RNA Sequencing shows aberrant Hippo expression

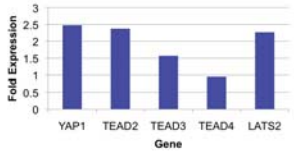


Figure 2. RNA Sequencing screen of the Hippo Pathway. Fold expression was calculated relative to a pediatric non-tumor brain sample.

Hippo Pathway is Turned Off in ATRT

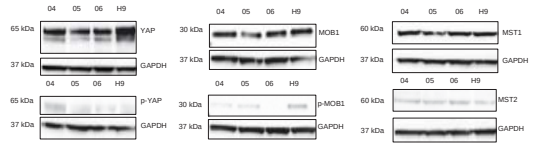


Fig. 3. Western blot analysis of endogenous protein expression of Hippo pathway components. GAPDH was used as a loading control.

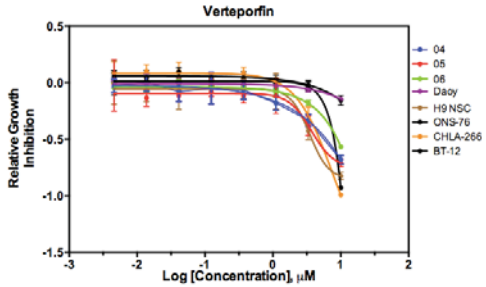


Figure 3. Drug-dose Response Curve for ATRT cells after Verteporfin treatment. Cells were seeded at a concentration of 2000/well, with 1/3^n dilutions ranging from 2uM to 4nM Verteporfin, and compared to a vehicle (DMSO) and kill control (MG142 proteosome inhibitor), and allowed 72 hours representing a full doubling cycle before being analyzed by CellTiter Glo assay. IC_{50} was calculated as the value required for 50% relative inhibition of available ATP in the cell lysate after 72 hours"

Conclusion

1. Despite aberrant expression in ATRT cells, there was no response with Verteporfin treatment

2. Small-molecule inhibition of the YAP/TEAD complex does not appear to be a viable therapeutic strategy for atypical teratoid rhabdoid tumor

Future Work

•Determine whether other Hippo pathway factors are viable therapeutic targets in the treatment of ATRT

•Determine if there is aberrant Hippo expression in other pediatric neoplasms

•Investigate the role of Hippo small-molecule inhibitors in the treatment of other pediatric neoplasms

References

1. Coccé MC, Lubieniecki F, Kordes U, Alderete D, Gallego MS. A complex karyotype in an atypical teratoid/rhabdoid tumor: case report and review of the literature. *Journal of Neuro-Oncology*. 2010;104(1):375-380.

2. Fernandez-L A, Northcott PA, Dalton J, et al. YAP1 is amplified and up-regulated in hedgehog-associated medulloblastomas and mediates Sonic hedgehog-driven neural precursor proliferation. *Genes & Development. 2009;23(23):2729-2741.*

3. Baia GS, Caballero OL, Orr BA, et al. Yes-Associated Protein 1 Is Activated and Functions as an Oncogene in Meningiomas. Molecular Cancer Research. 2012;10(7):904-913

4. Bhat KPL, Salazar KL, Balasubramaniyan V, et al. The transcriptional coactivator TAZ regulates mesenchymal differentiation in malignant glioma. Genes & Development. 2011;25(24):2594-2609.

5. Brodowska K, Moujahed A, Marmalidou A, et al. The clinically used photosensitizer Verteporfin (VP) inhibits YAP-TEAD and human retinoblastoma cell growth in vitro without light activation. *Experimental Eye Research*.

6. Piccolo S, Cordenonsi M, Dupont S. Molecular Pathways: YAP and TAZ Take Center Stage in Organ Growth and Tumorigenesis. *Clinical Cancer Research*. 2013;19(18):4925-4930.

7. Johnson R, Halder G. The two faces of Hippo: targeting the Hippo pathway for regenerative medicine and cancer treatment. Nat Rev Drug Discov. 2014;13(1):63-79.

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