

Overactivation of Histone Deacetylases in the Development of Cancerous Stem Cells in Wilms Tumorigenesis

Introduction

Nephroblastoma, commonly known as Wilms Tumor (WT), is the most common pediatric kidney cancer in the US. While there are excellent survival rates of WT, treatment plans fail in up to 15% of patients, with negative long-term effects of chemotherapy, radiation, and other treatments affecting an even higher percentage. Thus, it is crucial we continue studying the mechanisms of WT to develop novel therapies that will maintain the high remission rate while reducing the burden of treatments.

While there have been numerous advances in WT pathogenesis over recent decades, the mechanisms that lead to WT growth remain unclear. Epigenetic mechanisms have been a region of interest due to previous studies that show embryonic exposure to adverse environments correlate to the development of WT in children. Altered epigenetics is a crucial component in the development of numerous cancers because of its role in chromosomal and histone mutations that lead to unregulated cell proliferation, hence why studying epigenetic mechanisms is a promising manner to identify why WT is developed.

Specific epigenetic modulators, histone deacetylases 1 (HDAC1) and HDAC2, have proven to relate to tumorigenesis of numerous cancers, including WT, thus they are a promising epigenetic approach towards the prevention and treatment of WT.

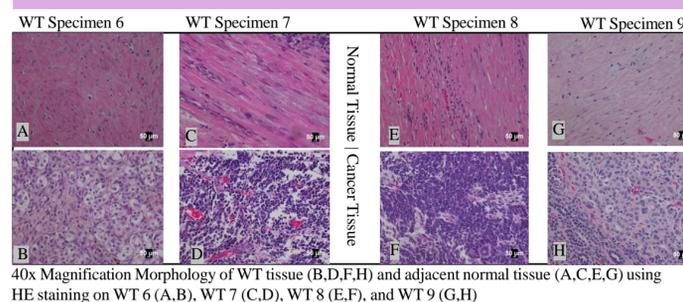
We hypothesize that the overactivation of HDAC1 and HDAC2 play critical roles in the initiation and progression of WT. The sine oculis (SIX) family are key regulators for developmental processes and tumorigenesis. A previous study showed that SIX1 and SIX2 (SIX1/2) are responsible for Wilms tumorigenesis. Moreover, SIX2 and CITED1 are the master genes regulating nephrogenesis. SIX2+CITED1+ cells have been identified as the nephrogenic-like cancer stem cells of WT, contributing to the resistance of vincristine, commonly used clinically for WT. We also identified the presence of early renal progenitor cells (SIX2+CITED1+) in human WT specimens and their association with overactivation of HDAC1/2.

Methods

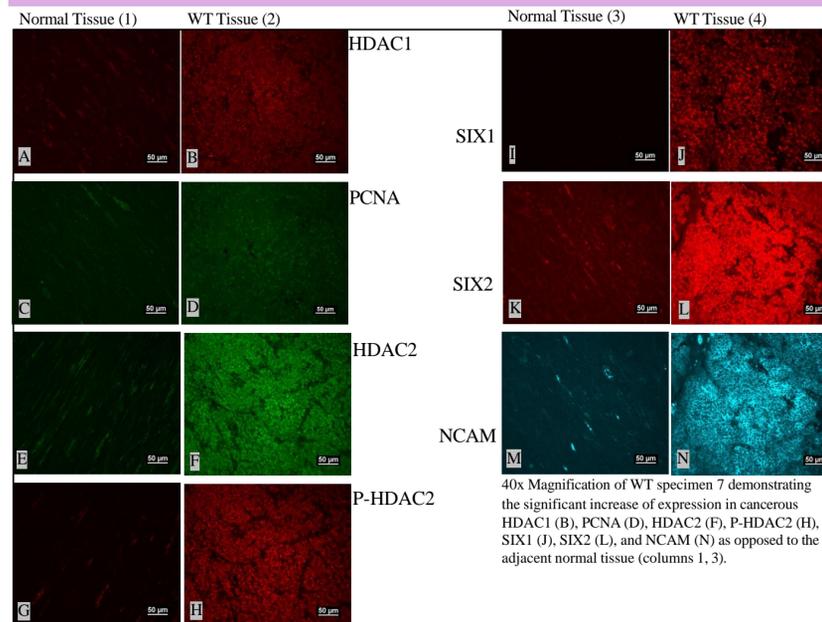
We have the approval from Tulane Human Research Protection Office; Institutional Review Boards (Study number: 2019-623) to study WT specimens. Four formalin fixed WT specimen with collection dates between 2013 and 2016 were analyzed using the following methods

- Hematoxylin and Eosin (HE) staining of each WT specimen were analyzed for morphologic evaluation and the comparison between cancerous tissue and adjacent normal tissue within each specimen.
- Immunostaining of HDAC1, PCNA, HDAC2, Phosphorylated HDAC2 (P-HDAC2), SIX1, SIX2, and NCAM were analyzed in each WT specimen to determine the level of expression in cancerous tissue as opposed to the adjacent normal tissue.
- Multicolor fluorescent in situ hybridization (RNAScope) combined with Immunofluorescence (IF) staining was used on two WT specimen to identify the presence of SIX2+CITED1+ cells in cancerous tissue in contrast to adjacent normal tissue.
- To study the regulation of SIX1/2 expression by HDAC1/2, HDAC1 and HDAC2 were overactivated in human embryonic kidney 293 (HEK 293) cells. The cells were transfected with Flag- HDAC1 and/or Flag-HDAC2, and HDAC2/SIX2 levels were evaluated by Western Blot (WB) analysis and quantified against the endogenous β -actin level.

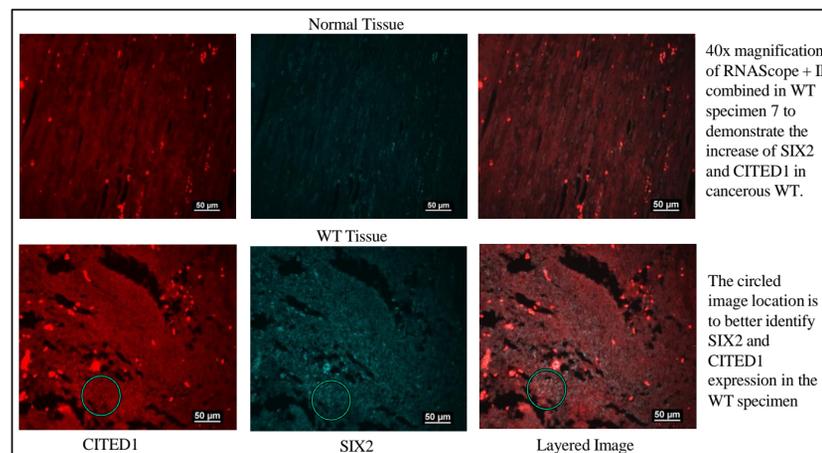
Morphology



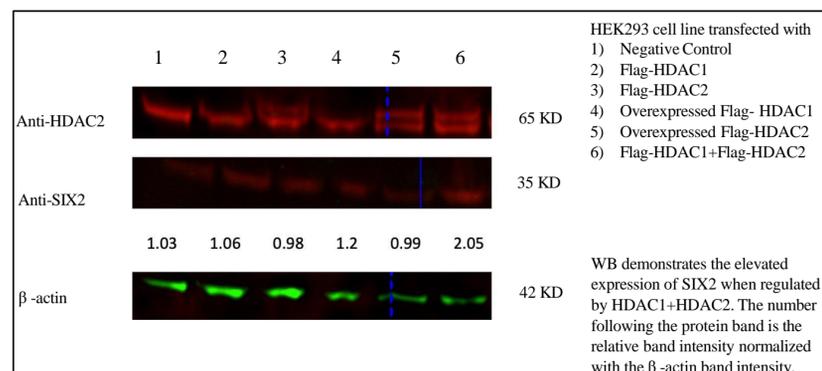
Immunostaining



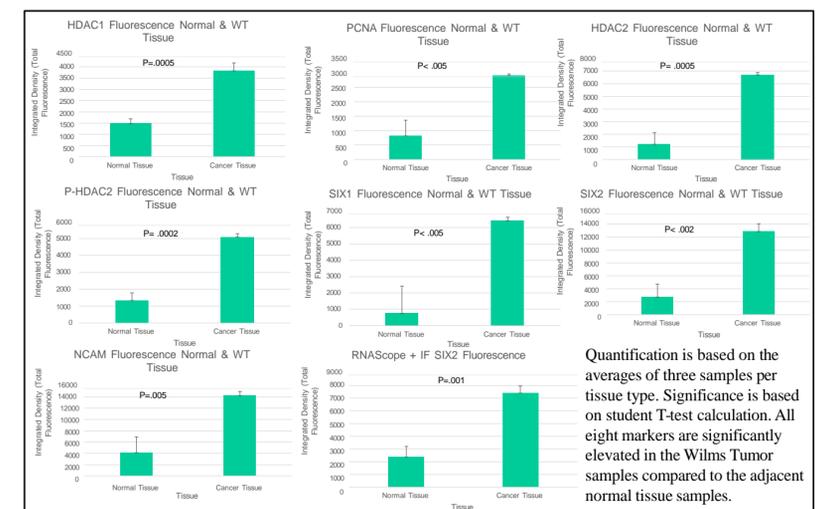
RNAScope + IF



Western Blot



Quantification



Results

Results of the HE staining reveal cancerous tissue to be a darker blue color with overlapping nuclei and a biphasic/triphasic appearance containing blastemal, epithelial, and stromal tumors. Adjacent normal tissue portrays a pink color with very linear structure.

The immunostaining demonstrates a significant increase in the expression of HDAC1, PCNA, HDAC2, P-HDAC2, SIX1, SIX2, and NCAM in WT tissue. This provides evidence that there is an overactivation of HDAC1 and HDAC2 in WT tissue, strongly suggesting the role of HDAC1 and HDAC 2 in Wilms tumorigenesis.

The RNAScope + IF shows a high expression of CITED1 and SIX2 in WT tissue as opposed to adjacent normal tissue, providing support that there is a significant increase of SIX2+CITED1+ cells in WT. Because of previous studies demonstrating the correlation between SIX2+CITED1+ cells and cancerous stem cell growth, being able to identify a higher level of SIX2 and CITED1 in WT cells will be a promising step towards connecting the overactivation of HDAC1/2 to the overexpression of SIX2 and CITED1. The regulation of SIX2 and CITED1 by HDAC1 and HDAC2 will support the hypothesis of HDAC1/2 playing a role in cancerous stem cell growth in WT.

The western blot results indicate the upregulation of SIX2 by overexpression of HDAC1/2. The SIX2 intensity (after being normalized with β -actin), is twice that of any other plasmid, illustrating that when HDAC1 and HDAC2 are both overexpressed, there will be a higher expression of SIX2.

Conclusion

In conclusion, our studies show the increased expression of HDAC1, HDAC2, SIX1, and SIX2 in cancerous tissue as opposed to the adjacent normal tissue, the increase in SIX2+CITED1+ cells in WT samples, and the regulation of SIX2 by HDAC1 and HDAC2 in the HEK293 cell line. Therefore, continued elucidation of HDAC1 and HDAC2 in WT pathogenesis and cancerous stem cell development will be a promising approach towards novel therapies to treat WT with the same excellent survival rates but lower toxicity level and negative long-term effects.

We will also study the regulation of CITED1 expression by HDAC1/2. The regulation of SIX2+CITED1+ cell formation by overactive HDAC1/2 will further provide evidence towards the development of cancerous stem cells in WT, potentially establishing a new target for future medicinal therapies.