

Annabelle L Bolitho
Undergraduate
Tulane University, New Orleans, Louisiana

Hongbing Liu, PhD:
Tulane School of Medicine, Department of Pediatric Nephrology

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

Nephroblastoma, commonly referred to as Wilms Tumor (WT), is a pediatric kidney cancer most typically affecting children ages 3 to 5, although older children and adults can still be diagnosed, only less frequently (1). While WT has a high remission rate, current treatment plans fail in approximately 15 percent of patients. Moreover, survivors suffer from both complications and the long-term effects of aggressive treatments (2). Thus, there is a crucial need to advance our understanding of tumorigenesis and develop novel therapies to reduce the hardship of treatments while maintaining excellent survival rates. Over recent decades, there have been numerous advances surrounding WT pathogenesis, however, the mechanisms that disrupt differentiation and lead to tumor growth are still unclear. WT is associated with certain birth defects and gene mutations, although there is no evidence supporting the direct genetic inheritance of cancer from parent to offspring. Studies have revealed the positive association between WT in children and embryonic exposure to adverse environments. Environmental effects are believed to be mediated by gene expression reprogramming, including epigenetic changes. Altered epigenetics is central to oncogenesis in a variety of pediatric cancers because of their role in chromosomal and histone mutations that lead to unregulated cell proliferation (3). An area of interest within epigenetic mechanisms lies in epigenetic modulators, histone deacetylases 1 (HDAC1) and HDAC2, which have been proven to relate to tumorigenesis in a variety of cancers, including WT (4). Thus, epigenetic approaches are promising for the prevention and treatment of WT.

To better understand the potential role of HDAC1 and HDAC2 in Wilms tumorigenesis, four WT specimen were studied, with collection dates ranging from 2013 to 2016. They were analyzed using hematoxylin and eosin (HE) staining, immunostaining of HDAC1, HDAC2, and SIX2, and multicolor fluorescent in situ hybridization (RNAScope) for CITED1 and SIX2. Previous studies have demonstrated a connection between SIX2+ CITED1+ cells to nephrogenic-like cancerous stem cells in WT (5). Furthermore, cultured 293T human embryonic kidney cells (HEK293), were transiently transfected with Flag-HDAC1 and Flag-HDAC2 expression plasmids, to determine their relevance to the expression of SIX2 and CITED1.

Each WT specimen demonstrated a biphasic or triphasic pattern with blastemal, stromal, and epithelial tumors within the cancerous tissue. As opposed to adjacent normal tissue, the malignant was portrayed by small blue dots and overlapping nuclei. Immunofluorescence (IF) demonstrated a significantly higher level of HDAC1, HDAC2, and P-HDAC2 (phosphorylated at Ser 394) in the tumor tissues compared with adjacent normal tissues, strongly suggesting that HDAC1/2 plays a role in Wilms tumorigenesis. Moreover, by combining RNAScope with IF, we promisingly demonstrated the existence of SIX2+ CITED1+ cells in human WT specimen and their linkage to overactivated HDAC1/2. Finally, we did an HDAC1/2 knockdown (loss of function) and HDAC1/2 overexpression (gain of function) in the HEK293 line to study the regulation of SIX1/2 expression by HDAC1/2. In conclusion, our study provides evidence for the association of high expression of HDAC1/HDAC2 with SIX2+ CITED1+ cancer stem cells in WT. Overactivation of HDAC1 and HDAC2 may be an important mechanism for the initiation and progression of WT.