THE GRADUATE COLLEGE OF THE
UNIVERSITY OF OKLAHOMA HEALTH SCIENCES CENTER

ANNOUNCES THE FINAL EXAMINATION OF

Eleni Petsouki

FOR THE DEFENSE OF THE DOCTOR OF PHILOSOPHY DEGREE
GRADUATE COLLEGE
Department of Cell Biology

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FBW7 promotes osteoblast differentiation by maintaining structural and functional integrity of primary cilia in mesenchymal stem cells

COMMITTEE IN CHARGE: *Leonidas Tsiokas, PhD; Dean Dawson, PhD; Mary Beth Humphrey, MD, PhD; Yuechueng Liu, PhD; James J. Tomasek, PhD*

ABSTRACT: The primary cilium is an antenna-like organelle functioning as a hub for several cellular pathways, including the Hedgehog pathway. Structural defects and/or changes in ciliary length result in diverse diseases/syndromes. However, whether and how changes in ciliary length affect signaling output is unclear. We examined this question in the context of stem cell differentiation. Mesenchymal stem/stromal cells (MSCs) reside in the bone marrow and generate different cell types including osteoblasts. While primary cilia are known to have an essential role in osteoblastogenesis, the mechanisms by which ciliary length control osteoblast differentiation have been largely unknown. A cellular pathway that controls ciliary length in differentiated cells, involves the SCF^{FBW7} E3 ubiquitin ligase, which mediates the destruction of NDE1, a negative regulator of ciliary length. Here, I specifically studied whether and how the FBW7-mediated degradation of NDE1 controls MSC differentiation to osteoblasts. First, I showed that FBW7 is required for the differentiation of MSCs to osteoblasts using complementary *in vitro*, *ex vivo* and *in vivo* approaches. *In vitro* and *ex vivo* studies showed that *Fbxw7*-null C3H10T1/2 cells and freshly isolated MSCs from the bone marrow of *Fbxw7*-null mice showed severely delayed osteoblastogenesis. Consistently, post-natal deletion of *Fbxw7* resulted in reduced bone mass and decreased levels of osteoblast-specific markers in the serum of 3 month old male mice. Mechanistic experiments in C3H10T1/2 cells revealed that FBW7 controls the abundance of both positive (TALPID3) and negative regulators (NDE1) of ciliary length. Furthermore, both TALPID3 and NDE1 positively regulate the Hedgehog pathway via distinct mechanisms, while excessive Hedgehog activity suppresses the expression of TALPID3 via a negative feedback loop. This network of proteins and their positive and negative interactions maintain a tight coupling between ciliary length and Hedgehog signaling output that is essential for osteoblast differentiation. Given that FBW7 is one of the most commonly mutated tumor suppressors, TALPID3 a gene mutated in Joubert syndrome, and NDE1 a microcephaly gene, my studies further suggest that seemingly unrelated diseases may share a common pathomechanism of failing to couple ciliary structure with function.