UNIVERSITY of GUELPH

COLLEGE of BIOLOGICAL SCIENCE

DEPARTMENT OF MOLECULAR AND CELLULAR BIOLOGY

Announcement:

All interested members of the university community are invited to attend the Final Oral Examination for the degree of ***Doctor of Philosophy*** *of*

HUI ZHU

On Wednesday, December 6, 2023 at 9:30 a.m. (SSC 2315)

Thesis Title: **Transgenic models to characterize the role of Dlx5 in endochondral ossification**

Examination Committee:

Dr. Yang Xu, Dept. of Molecular and Cellular Biology (Exam Chair)
Dr. Andrew Bendall, Dept. of Molecular and Cellular Biology
Dr. Terry Van Raay, Dept. of Molecular and Cellular Biology
Dr. Nina Jones, Dept. of Molecular and Cellular Biology
Dr. Aimee Ryan, Depts. of Pediatrics and Human Genetics, McGill University (External Examiner)

Advisory Committee:
Dr. Andrew Bendall (Advisor)
Dr. Ray Lu
Dr. Terry Van Raay

Abstract: Results from both loss-of-function and misexpression studies have implicated Dlx5 and Dlx6 as partially redundant positive regulators of chondrocyte hypertrophy in the appendicular skeleton. To date, however, it has not been possible to conclude whether these effects are cell autonomous. To address this question, we first engineered transgenic mice in which Dlx5 expression is specifically restricted to immature and differentiating chondrocytes, and not in the perichondrium. The Col2a1-Dlx5 transgene was expressed in the same pattern as endogenous Col2a1. Col2a1-Dlx5 transgenic embryos and neonates display accelerated chondrocyte hypertrophy and mineralization throughout the endochondral skeleton, the severity of which varied in proportion to transgene copy number. In a parallel study, the chicken Dlx5 gene was epitope-tagged and cloned downstream of Col2a1 regulatory sequences then inserted into the retroviral vector RCANBP(A). RCAN-Col2-Dlx5 viral particles were injected unilaterally into limb buds of chicken embryos. Immunohistochemistry with anti-Flag antibody indicated the expression of exogenous Dlx5 in chondrocytes. The mineralization of long bones in infected wings was more advanced compared to the contralateral control wing when measured ten days post-infection. To assess the function of Dlx5 in the cartilaginous anlagen of the endochondral bones in the absence of Dlx5 expression in other murine tissues, we introduced this allele onto a Dlx5/6 null background. Defects of chondrocyte differentiation characteristic of the Dlx5/6 null phenotype were specifically rescued in Dlx5/6-/-; Col2a1-Dlx5 embryos and neonates. Together these results lead us to conclude that the role of Dlx5 in the hypertrophic pathway is cell autonomous. These murine embryos further demonstrate functional equivalency between Dlx5 and Dlx6 in the endochondral skeleton, in that the requirement for Dlx5 and Dlx6 function can be satisfied with Dlx5 alone.

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Curriculum Vitae: Hui completed her Master of Science in Molecular Biology and Genetics at the University of Guelph in 2004. She began her PhD studies in Molecular Biology and Genetics from 2005 to 2009. She went on to complete her Diploma in Medical Laboratory Technology at Mohawk College in 2014. She then returned to her PhD studies at the University of Guelph in 2023.

Publications: **Zhu H**, Bendall AJ. (2009) Dlx5 Is a cell autonomous regulator of chondrocyte hypertrophy in mice and functionally substitutes for Dlx6 during endochondral ossification. *PLoS One* 4(11)

Zhu H, Bendall AJ. (2006) Dlx3 is expressed in the ventral forebrain of chicken embryos: implications for the evolution of the Dlx gene family. *Int J Dev Biol.*50 (1):71-5.

Hsu SH, Noamani B, Abernethy DE, **Zhu H**, Levi G, Bendall AJ.(2006) Dlx5- and Dlx6-mediated chondrogenesis: Differential domain requirements for a conserved function. *Mech Dev.*123(11):819-30.