Poster Number

51 **sPDZD2: A Novel Negative Modulator of Hedgehog Signaling**

Tsui M., Chan D., Cheah K., Cheung M. and Yao K.-M.
Department of Biochemistry, The University of Hong Kong

PDZD2 is a multi-PDZ domain-containing protein of unknown function in early development. It is proteolytically cleaved to generate its secreted form, sPDZD2. Human PDZD2 is mapped to chromosome 5p13.2, which co-localizes with the disease-associated gene in a family of Brachydactyly Type A1 (BDA1) patients, suggesting involvement of PDZD2 in limb development. Hedgehog (Hh) is an important morphogen that dictates tissue patterning during embryonic development and recent studies showed that mutations in Indian Hedgehog (IHH) resulted in BDA1. Interestingly, in situ hybridization revealed that Pdzd2 was expressed in the distal mesenchyme partially overlapping with Shh in mouse limb bud. During digit patterning, Pdzd2 was expressed in the interzone that flanked the Ihh/Gli1-expressing phalanx condensation. Moreover, Pdzd2 was expressed in the paraxial mesoderm adjacent to the differentiating neural tube. It is worth noting that PDZD2 protein was detected at the neural tube away from its site of synthesis, indicating a non-cell autonomous role of PDZD2 possibly via sPDZD2. Pdzd2 expression in various Hh-active tissues in mouse and chicken suggested an evolutionary conserved role of Pdzd2 in modulating general Hh signaling during early development.
Functional studies showed that overexpression of sPDZD2 in the chicken neural tube leads to down-regulation of NKX2.2 and OLIG2 expression. sPDZD2 was shown to counteract the ectopic NKX2.2 expression induced by long-range signaling of ectopic HH. Consistently, sPDZD2 exhibited an inhibitory effect on SHH-induced reporter activity in a Gli-luciferase cell line. Taken together, our results provided the first evidence that sPDZD2 is a negative modulator of Hedgehog signaling.

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52 **Sufu and Gli3 repressor mediate the temporal basal-to-apical progression of hair cell differentiation in mammalian cochleae**

Boshi Wang[1], Elaine Y. M. Wong[1], Yuchen Liu[1], Chi-Chung Hui[2] and Mai Har Sham[1]
1 Department of Biochemistry, The University of Hong Kong, Pokfulam, Hong Kong, China
2 Program in Developmental and Stem Cell Biology, The Hospital for Sick Children, Toronto, Canada

The Sonic Hedgehog pathway plays important roles in mammalian inner ear development. Mutations of Shh, Smo and Gli3 lead to severe defects in mouse inner ear morphogenesis. However, knockout of Gli2 does not affect inner ear morphology or cochlear hair cell differentiation, suggesting that the Gli repressor function may be required for Hedgehog signaling during inner ear development. Sufu is a negative regulator of Hedgehog signaling and it functions to repress Gli activator and enhance Gli repressor activities. To evaluate the involvement of Sufu and Gli transcription factors in mediating cochlear hair cell differentiation, we have analyzed the Pax2Cre;Sufuflox/flox, Gli3P1-4/P1-4 and Gli3Δ699/Δ699 mutants using hair cell marker Myosin7a and supporting cell markers Sox2, P75 and Jag1. At E16.5, only one row of inner hair cells could be observed at the basal region of cochleae in the Pax2Cre;Sufuflox/flox mutants. Nevertheless, normal hair cells appeared at the medial region at E18.5, indicating that deletion of Sufu delays cochlear hair cell differentiation. Gli3 repressor is abolished in the Gli3P1-4/P1-4 mutant, in which cochlear hair cell differentiation was delayed. Interestingly, in the Gli3Δ699/Δ699 mutant with excessive Gli3 repressor, hair cell differentiation was accelerated in the apical region of the cochlear duct. Our results suggest that Sufu and Gli3 repressor are essential factors which regulate the temporal basal-to-apical progression of cochlear hair cell differentiation, supporting that Sonic Hedgehog signaling is required to control the dynamics of hair cell differentiation.