Antagonistic crosstalk of Wnt/β-catenin/Bmp signaling within the Apical Ectodermal Ridge (AER) regulates interdigit formation

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Interdigital cell death (ICD) is an essential event associated with the individualization of digits in the developing limb. Bmps, *Fgf8* and Retinoic acid (RA) have been previously known as important mediators to control the progress of ICD in the limb development. Although several molecules have been identified to participate in the regulation of ICD, the mechanism of action and their crosstalk that contribute in the process of ICD remains unknown. Wnt/β–catenin signaling is an important factor that specifies and regulates the formation of many tissues and organs. Ablation and stabilization of Wnt/β–catenin leads to several organ dysmorphogenesis and the onset and progression of cancer formation. Previous studies have suggested the involvement of Wnt/β-catenin signaling in the induction and maintenance of the apical ectodermal ridge (AER) during limb development. The AER is a transient specialized epithelium at the distal limb tip which is considered as essential for the embryonic limb outgrowth along the proximodistal axis. However, the importance of Wnt/β-catenin signaling in the process of ICD and in the digital and interdigital formation is still unclear. Thus, this study investigated the involvement of Wnt/β–catenin signaling as an essential factor to regulate digit and interdigit formation and its crosstalk with the other signaling pathways. To determine the importance of Wnt/β–catenin signaling in the regulation of digit and interdigit formation, the gain of function (GOF) of β–catenin allele mouse was utilized. Keratin 5 (K5) Cre (Cre recombinase) was used

as a Cre driver mice to stabilize β–catenin in the AER and in the surface ectoderm of the limb. The phenotype of the K5Cre-Catnb$^{(ex3)fl/+}$ shows digital fusion and failure of cell death to occur in the interdigit. ICD in the interdigital region of the mutant limb was reduced compared with the control. Furthermore, addition of mutation in one of the essential Bmp receptor, *Bmpr1a*, induces more severe digital fusion than that of K5Cre-Catnb$^{(ex3)fl/+}$ mutant embryos. These results suggest that Wnt/β–catenin locates genetically upstream of *Fgf8*. These results add to the growing evidences of interplay of growth factor signaling to regulate ICD in the AER. Moreover, elucidation of the mechanisms behind the proper formation and individualization of digits will help to understand the etiology of congenital anomalies of the limb.