THE EXPRESSION OF FGF8, BMP4 AND SHH ASSOCIATED WITH CENTRAL POLYDACTYLY, SYNDACTYLY AND CLEFT FOOT INDUCED IN RAT EMBRYOS

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Introduction: Various combinations of central polydactyly, syndactyly, and cleft hand have been frequently observed in the individual hands and feet in the same patients. In addition, experimental studies have shown that these differences and their various combinations can be induced simultaneously in rat embryos whose pregnant mothers have been treated with a teratogen at a critical time. Based on these facts, central polydactyly, syndactyly, and cleft hand have been classified into a single category, abnormal induction of digital rays during limb development. The aim of this study was to detect abnormal localization of cell death and abnormal expression of fibroblast growth factor 8 (Fgf8), bone morphogenetic protein 4 (Bmp4), and Sonic hedgehog (Shh), and to demonstrate the pathway through which central polydactyly, syndactyly, and cleft foot.

Methods: Inbred WKA/Hkm rats were used. Pregnant females at embryonic day (E) 11 were given a single oral dose of 20 mg/kg busulfan. Busulfan is used to treat chronic myelocytic leukemia. The control and treated embryos from E12 to 17 were removed and processed by the following methods. Hindlimbs were observed. A total of 179 pregnant females and 832 embryos were used, and 1112 hindlimbs were observed in this study. Detection of cell death: To identify areas of cell death in hindlimbs, whole embryos from E12 to 17 were stained with Nile blue (NB) sulfate. Clusters of dead cells were used, and 1112 hindlimbs were observed in this study. Whole-mount in situ hybridization: Whole-mount in situ hybridization for embryos at E12 to 14 was performed. Antisense RNA probes for mouse Fgf8, Bmp4 and Shh were used for in situ hybridization.

Results: Localization of cell death: In control embryos, programmed cell death (PCD) was detected in the AER from E12 to 14. PCD was detected in the interdigital mesenchyme subjacent to the AER at E15. By E16, PCD was detected in the AER and whole area of the mesenchyme from E12 to E14. By E15, abnormal clefts in the central part of the footplate were observed. Cell death was detected in the anterior and posterior mesenchyme at E15. PCD of the mesenchyme as shown in control embryos at E15 and E16 was not detected in the central part of the footplates in treated embryos (Fig. 1). Fgf8 expression: In control and treated embryos, Fgf8 expression was detected in the AER from E12 to 14. Comparison of control and treated embryos showed that Fgf8 expression at E13 and E14 was reduced in treated embryos (Fig. 2). Bmp4 expression: In control and treated embryos, Bmp4 expression was detected in the AER and distal part of the mesenchyme at E12 and E13. Comparison of control and treated embryos showed that Bmp4 expression at E13 was reduced markedly in the distal part of the mesenchyme in treated embryos (Fig. 3). Shh expression: In control and treated embryos at E13, Shh was expressed in the AER and whole area of the mesenchyme. No differences of Shh expression between control and treated embryos were detected at E13 (Fig. 4).

Discussion: FGF8 promotes limb outgrowth, and reduction of FGF8 expression may reduce cell death and result in abnormal clefts. Severe abnormal cleft seemed to lead to cleft foot. BMP4 controls cell death and reduction of Bmp4 may affect the potential to condense and to form extra digits. Therefore, reduction of Bmp4 expression can induce abnormal expression of Bmp4 in control and treated embryos. This suggests that Bmp4 signaling plays an important role in centrally organizing digit formation. In this study, there were no differences in Shh expression between control and treated embryos. This indicates that Shh signaling does not function in central digit formation. We suggest the following pathway for central polydactyly, syndactyly, and cleft foot: reduction of Fgf8 expression results in reduction of limb outgrowth and abnormal cleft. Reduction of Bmp4 expression causes undifferentiated mesenchymal cells to escape from PCD and to induce interdigital web and extra digits with Shh signaling. If both Fgf8 and Bmp4 are reduced, Shh signaling can induce abnormal digital rays, including central polydactyly, syndactyly, cleft foot and their various combinations. The proportion of FGF8 and BMP4 may play an important role in determining the future phenotype, central polydactyly, syndactyly, cleft foot or their various combinations.

![Figure 1: Localization of cell death at E16. (A) In control embryos, PCD was detected in the interdigital tissues (arrowheads). (B) In treated embryos, PCD was not detected in the area of abnormal clefts (arrow).](image1.png)

![Figure 2: Expression of Fgf8 at E13. (A) In control embryos, Fgf8 expression was detected in the AER (arrowheads). (B) In treated embryos, Fgf8 expression in AER was reduced (arrowheads).](image2.png)

![Figure 3: Expression of Bmp4 at E13. (A) In control embryos, Bmp4 expression was detected in the AER (arrowheads) and distal part of the mesenchyme (arrows). (B) In treated embryos, Bmp4 expression was severely reduced in the mesenchyme (arrows).](image3.png)

![Figure 4: Expression of Shh at E13. (A, B) In control and treated embryos, Shh expression was detected in the posterior mesenchyme (arrowheads).](image4.png)

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