Introduction: Tibial ray deficiencies, human abnormalities of the lower leg, belong to longitudinal deficiency. We induced tibial ray deficiencies in rat embryos by treating the mother with teratogen at a critical time (1, 2). These deficiencies are related closely to a deficit of mesenchymal cells in the limb buds caused by the impairment before the formation of the limb bud. However, little is known about the early changes of tibial ray deficiencies 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), Homeobox (Hox) a11, Hoxd11 and Sonic hedgehog (Shh), and to demonstrate the pathway to tibial ray deficiencies.

Methods: Inbred WKAl/Hkem rats were used. Pregnant females at embryonic day (E) 10 were given a single oral dose of 20mg/kg busulfan. Busulfan is used to treat chronic myelocytic leukemia. The control and treated embryos from E12 to 14 were removed and processed by the following methods: Detection of cell death: To identify areas of cell death in hindlimbs, whole embryos from E12 to 14 were stained with Nile blue (NB) sulfate. Clusters of dead cells phagocytized by macrophages were shown as blue granules stained with NB sulfate. 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, Hoxa11, Hoxd11 and Shh were used for in situ hybridization.

Results: Localization of cell death: In treated embryos, cell death was detected in the whole area of the mesenchyme from E12 to 14 (Fig. 1). Fgf8 expression: In control and treated embryos, Fgf8 expression was detected in the AER from E12 to 13, in the AER and distal part of the mesenchyme at E14. Comparison of control and treated embryos showed that Fgf8 expression at E12 and 13 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 to 14. Comparison of control and treated embryos showed that Bmp4 expression at E12 to 14 was reduced in the AER and the distal part of mesenchyme in treated embryos. Especially in the anterior part of mesenchyme, Bmp4 expression was markedly reduced (Fig. 3). Hoxa11 expression: In control and treated embryos at E12 to 14, Hoxa11 was expressed in the distal part of mesenchyme. No differences of Hoxa11 expression between control and treated embryos were detected. Hoxd11 expression: In control and treated embryos at E12 to 14, Hoxd11 was expressed in the distal part of mesenchyme. No differences of Hoxd11 expression between control and treated embryos were detected. Shh expression: In control and treated embryos at E13, Shh was expressed in the posterior part of mesenchyme. No differences of Shh expression between control and treated embryos were detected at E13.

Discussion: Cell death in the limb buds results in reduction of the number of mesenchymal cells. That may cause skeletal hypoplasia. FGF8 promotes proliferation of mesenchymal cells and limb outgrowth (3). Reduction of Fgf8 expression results in reduction of the proliferation of mesenchymal cells and reduction of limb outgrowth. That may cause skeletal hypoplasia. BMP4 promotes proliferation of mesenchymal cells and controls differentiation (4, 5). Reduction of Bmp4 expression results in reduction of the proliferation of mesenchymal cells and delay of differentiation. Especially in the anterior part of mesenchyme, Bmp4 expression was markedly reduced. Reduction of Bmp4 expression in the anterior part of mesenchyme may cause skeletal hypoplasia in the area where tibial ray will be formed. In this study, there were no differences in Hoxa11, Hoxd11 and Shh expression between control and treated embryos. Cell death in the limb buds, reduction of Fgf8 expression and Bmp4 expression may cause skeletal hypoplasia. Tibial hypoplasia may cause tibial ray deficiencies.

References

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