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MINI REVIEW

Our challenging to understand non-genetic effect in addition to genetic one on dento-craniofacial morphogenesis in spontaneous cleft lip/palate mouse model from the standing point of pediatric dentistry

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Summary Cleft lip with or without cleft palate is the most common congenital anomaly in craniofacial complex, which is our target to deepen our understanding toward a molecular mechanism of dento-craniofacial morphogenesis. The hypothesis that the maternal effects in addition to the genetic ones play important roles on the dento-craniofacial morphogenesis in mice has been tested based on each developmental stage. Firstly, the maternal effects on the intrauterine craniofacial development in the mouse fetus were examined by means of embryo transfer technique, skeletal staining and cephalometry, indicating that the maternal effects were one of important factors on the intrauterine craniofacial morphogenesis of CL/Fr mice. Secondly, when the molecular nature of the maternal effects is elucidated, maternally derived growth factors may play important roles on mouse fetus development. Bone morphogenetic protein 4 (BMP4) and epidermal growth factor (EGF) function antagonistically, yet are coupled in the regulation of initial chondrogenesis. Smad1 serves as a point of convergence for the integration of two different growth factor signaling pathways during chondrogenesis in the mouse fetal mandible. Lastly, transforming growth factor-beta3 (TGF-β3) promoted fusion of cleft lip in the mouse fetus through the molecular pathways. In fact, microsurgical repair of cleft lip in the fetus that produced scarless fusion is mediated by TGF-β3 regulation of mesenchymal cell proliferation and migration at the site of repair. In addition, TGF-β3 promoted cell proliferation and angiogenesis in lip mesenchymal tissues. These events lead to enhancement of the lip fusion in the presence of TGF-β3. In neonatal mouse, application of exogenous TGF-β3 to decrease type I collagen accumulation and consequential scar formation provide the opportunities for the clinical augmentation of scar reduction after cleft lip repair. These findings indicate that the environmental factors provided by the dams cannot be ignored in the etiology of a craniofacial anomaly and/or development in the mouse model in addition to genetic ones.

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In mammalian, craniofacial development of the offsprings is influenced by its own genotype, maternal effects and non-maternal environmental factors. The maternal effects are composed of the prenatal and postnatal effects from dams. The prenatal effects are mainly due to the intrauterine condition of dams while the postnatal ones are mainly due to the breast feeding ability and nursing of dams (Fig. 1). In the mouse model, the prenatal effects of dams on the intrauterine dento-craniofacial development in the mouse fetus were examined by means of the embryo transfer technique [1], while genetic effects on dento-craniofacial development were studied using twin mice production [2]. Furthermore, the postnatal maternal effects on offspring development can be distinguished from the prenatal maternal ones by a cross-nursing method of dams. For example, a mutual exchange of a half of the litters between two strains of dams enables us to examine the inter-nursing difference of the postnatal development of the same genotype offspring derived from either the self-nursing or the cross-nursing dam [3].

Dento-craniofacial morphogenesis is greatly dependant on migration, proliferation and differentiation of cranial neural crest cells [4,5]. Cross talking between cell-to-cell and cell-to-extra-cellular matrix including basement membrane are always involved through craniofacial morphogenesis. Furthermore, complicated interactions continuously occur at the levels of the ligands, receptors, cytoplasmic transducers, transcription and translation processes followed by post-translational processes involving protein glycosylation, phosphorylation, and proteolysis. Cleft lip and palate (CLP) is one of major neurocriptopathies caused by abnormal migration, proliferation or differentiation of cranial neural crest cells and the occurrence rate of CLP is 1:700 in Orientals. CL/Fr mice strain, which has almost 25—35% spontaneous occurrence rate of CLP, gives us the good opportunities to examine the genetic, maternal and non-maternal environmental effects from the fetal to neonatal stages on CLP onset [6,7]. Epidermal growth factor (EGF) is considered to be predominately supplied to fetuses or newborns from the mothers through their placenta, amniotic fluid or breast feeding because of its poor gene expression level in the mice fetal tissues. Therefore, many maternally derived growth factors might play important roles in dento-craniofacial morphogenesis through cartilage formation in the mouse fetus [8]. However, detailed molecular mechanisms of the dento-craniofacial morphogenesis or development by the maternal effects at the prenatal and postnatal stages are still unknown. The purpose in this review is to focus on molecular dissection of the maternal influences on the CLP occurrence and the dento-craniofacial development from the fetal stage to young adulthood. Serial challenges including experimental techniques in embryology as well as molecular biology have been introduced at each developmental stage. These challenges could contribute to the molecular dissection of maternal effects during the fetal period in addition to genetic ones on the dento-craniofacial development of the fetus and newborn, and to providing the opportunities for exploring the prevention and molecular therapy against human craniofacial anomaly in future.

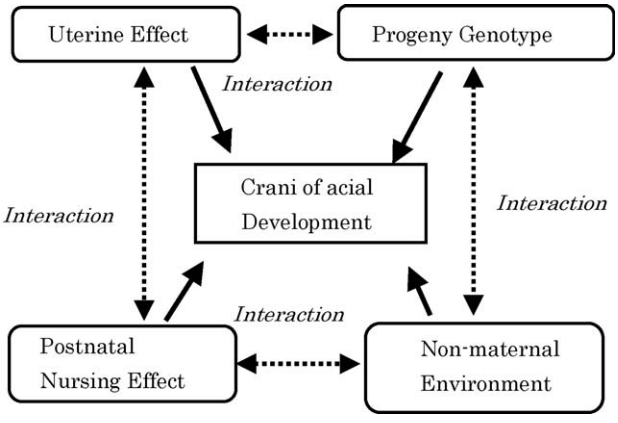

Figure 1 Development of offspring in mammalian.

1. The effects of dams on the craniofacial morphogenesis in CL/Fr mice fetuses

Well-developed CL/Fr blastocysts are surgically transferred into the pseudo-pregnant uteri of two kinds of the recipient mice strain, CL/Fr and C57BL females, by the microcapillary technique [9]. The dams were taken out from each recipient by laparatomy on the 18th gestational day to investigate the spontaneous incidence of CLP and the craniofacial structure change. The following results were obtained: (1) The CLP frequency was significantly higher in dams derived from CL/Fr recipient than C57BL. (2) The conditions of CL/Fr recipient-derived fetuses with CLP were significantly more serious than C57BL recipient-derived ones. (3) The overall craniofacial sizes of the unaffected fetuses from CL/Fr recipient were significantly smaller than those from C57BL recipient. Those of the affected dams from CL/Fr recipient were smaller than those from C57BL recipient although the inter-strain difference was not significant. (4) The dam strains had highly significant effects on the craniofacial size of the unaffected fetuses. These results suggested that prenatal effects from the dams played important roles on the incidence of CLP in CL/Fr mice fetuses.

2. Convergence of the BMP and EGF signaling pathways on Smad1 in the regulation of chondrogenesis

It is hypothesized that bone morphogenetic protein 4 (BMP4) and EGF mediated intracellular signals are both coupled in the regulation of Meckel's cartilage development. To investigate the reciprocal effects of BMP4 and EGF during chondrogenesis, the organ culture system of the mouse mandibular process and micromass culture system for chicken embryonic mandibular process were performed [10]. When BMP4- and EGF-soaked beads were implanted in juxtapositions of mouse mandible processes within embryonic day 10, the incidence and amount of ectopic cartilage, and Sox9 and type II collagen expression were significantly reduced and increased by BMP4 and EGF, respectively, in a dose-dependent manner. Similarly, in chicken serum-free micromass cultures, expression of constitutively active BMP receptor type IB by replication competent avian retrovirus system promoted the rate and extent of chondrogenesis; however, exogenous EGF attenuated these effects. In micromass cultures, BMP signaling resulted in nuclear translocation and accumulation of the signaling