Fibroblast growth factor-10 serves a regulatory role in duodenal development


Department of Pediatric Surgery, Developmental Biology Program, Children's Hospital Los Angeles, 4650 Sunset Boulevard, Saban Research Building 524, Mail stop#100, Los Angeles, CA 90027, USA

Abstract

Purpose: Duodenal obstruction occurs in 1 of 6000 live births and requires urgent surgical intervention. Duodenal atresia previously has been ascribed to a developmental failure of luminal recanalization; however, the cause of duodenal atresia remains incompletely understood. Although familial intestinal atresias have been described and syndromic associations are known, no specific genetic link has been established. Fibroblast growth factor-10 (Fgf10) is a known regulatory molecule relevant to mesenchymal-epithelial interactions, and mice deficient in Fgf10 demonstrate congenital anomalies in several organ systems including the gastrointestinal tract. The authors hypothesized that Fgf10 could serve a regulatory role in establishing normal duodenal development.

Methods: Wild-type mice with β-galactosidase under the control of the Fgf10 promoter were harvested from timed-pregnancy mothers. The expression of Fgf10 in the duodenum during development was evaluated by developing the embryos in X-Gal solution. Wild-type and mutant Fgf10/C0/C0 embryos were harvested from timed-pregnancy mothers at 18.5 days postconception (near term) and were analyzed for duodenal morphology (Institutional Animal Care and Use Committee–approved protocol 32-02). Photomicrographs were reviewed.

Results: Fibroblast growth factor-10 is active in the duodenum at a late stage of development. The Fgf10/C0/C0 mutants demonstrate duodenal atresia with a variable phenotype similar to clinical findings. The duodenum fails to develop luminal continuity and has proximal dilation. The phenotype occurs in an autosomal recessive pattern with incomplete penetrance (38%).

Conclusions: Fibroblast growth factor-10 serves as a regulator in normal duodenal growth and development. Its deletion leads to duodenal atresia and challenges traditionally accepted theories of pathogenesis. This novel, genetically mediated duodenal malformation reflects an animal model that will allow further evaluation of the pathogenesis of this surgically correctable disease. By studying the mechanism of Fgf10 function in foregut development, the authors hope to better understand these anomalies and to explore possible therapeutic alternatives.

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Although considerable effort has been put into investigating the organogenesis of the duodenum and many substantial theories have been offered, little has changed since Tandler [1] published his study of duodenal development in 1902. In that article, he reviewed the proposed causes of duodenal atresia at that time.

Wiederhofer describes a tumor obstructing the gut lumen as the cause for atresia. Forer blames it on deficient secretion of gall fluid, Preuss even on psychic disturbance of the mother, Hess on pressure exerted by the mesocolon. Ahlfeld thinks that the atresia he observed was caused by a persisting ductus omentum. Other authors variously blame it on an inguinal hernia, on omental artery, amniotic threads or thrombosis of the superior mesenteric artery. The most frequently given reason for atresia are the turning of the embryonic axis and peritonitis. Some authors maintain that peritonitis may occur in the fetus without leaving the usual traces, so that even in the absence of such signs, peritonitis can be considered as etiological agent. Other authors consider peritonitis not as the primary vent, but secondarily caused by the atresia, whereas others doubt that peritonitis can lead to atresia. In the same way, some authors doubt that turning of the embryonic axis could lead to atresia. Even accepting this possibility, the published material offers only few cases where the embryo has turned in a way that it might have lead to atresia. How can the turning of the axis in the region of the jejun-ileum be regarded as etiological factor for the atresia of the duodenum?...I believe that peritonitis as well as axis turning of the gut can be regarded as causes for duodenal atresia only in rare cases, if ever.

With this background in mind, Tandler meticulously studied 11 embryos and theorized that the normal solid cord stage of duodenal development and the failure of recanalization led to duodenal atresia. Many of the aforementioned ideas have been discarded, but the theory of an ischemic injury causing intestinal atresia has persisted, although more as an explanation of jejunoileal atresias. Louw and Barnard [2] proposed their theory of atresias resulting from a vascular accident in 1955. Nixon and Tawes [3] countered Barnard [2] proposed their theory of atresias resulting from an ischemic event, leading to duodenal atresia. Many of the aforementioned authors variously blame it on an inguinal hernia, on omental artery, amniotic threads or thrombosis of the superior mesenteric artery. The most frequently given reason for atresia are the turning of the embryonic axis and peritonitis. Some authors maintain that peritonitis may occur in the fetus without leaving the usual traces, so that even in the absence of such signs, peritonitis can be considered as etiological agent. Other authors consider peritonitis not as the primary vent, but secondarily caused by the atresia, whereas others doubt that peritonitis can lead to atresia. In the same way, some authors doubt that turning of the embryonic axis could lead to atresia. Even accepting this possibility, the published material offers only few cases where the embryo has turned in a way that it might have lead to atresia. How can the turning of the axis in the region of the jejuno-ileum be regarded as etiological factor for the atresia of the duodenum?...I believe that peritonitis as well as axis turning of the gut can be regarded as causes for duodenal atresia only in rare cases, if ever.

Cragan et al [6] quantified the higher prevalence of duodenal atresia among twins. Gahukamble et al [7,8] have reported on 2 families affected by duodenal atresia and suggested an autosomal recessive inheritance pattern in both. The association of duodenal atresia with other congenital malformations further supports the importance of genetics in the pathogenesis of duodenal atresia. Nixon [5] summarized known associated disorders: annular pancreas, Down’s syndrome, malrotation, and congenital heart defects. Other associated anomalies have been identified by Al-Salem [9], Lembrecht and Kluth [10], Annerén et al [11], and Sencan et al [12]. As momentum has increased for a genetic cause for duodenal atresia, Doray et al [13] and Gonzalez et al [14] have mapped specific cases of duodenal atresia to particular genes. The following is our proposed genetic mechanism for duodenal atresia.

1. Materials and methods

1.1. Mutant embryos

Young adult transgenic nlacZ-fibroblast growth factor-10 (Fgf10) mice (expressing β-galactosidase under the control of the Fgf10 promoter) were generated as described by Kelly et al [15]. Embryos were harvested late in development from timed-pregnancy mothers. The upper gastrointestinal (UGI) tract (including the distal esophagus, stomach, duodenum, proximal jejunum, and cystic duct) was harvested and fixed in 4% paraformaldehyde solution in phosphate-buffered saline for 2 hours. It was then incubated in X-Gal solution. Cells expressing Fgf10 stained blue on exposure to X-Gal. Photomicrographs were taken.

The Fgf10-null mice were generated in the C57/Bl6 murine strain by inserting a neomycin cassette into the first codon of the Fgf10 gene, as described by Sekine et al [16]. The Fgf10 null (−/−) indicates a homozygous deletion of the Fgf10 gene) mutant embryos died at birth because of the lack of lung development [16]. Wild-type (Wt) C57/Bl6 littermates were used as control subjects. Embryos were harvested near term (18.5 days postconception) from timed-pregnancy mothers. The UGI tract (including the distal esophagus, stomach, duodenum, proximal jejunum, and cystic duct) was harvested and photomicrographs were taken.

2. Results

Homozygous deletion of Fgf10 results in duodenal atresia with a 38% penetrance. The phenotype is predominantly that of a type III atresia [17] (complete separation), although type I atresias [17] are occasionally seen. This phenotype is inherited in an autosomal recessive pattern and is one of several severe developmental defects. (Sekine et al [16] detail some of these defects.)

Fig. 1 shows the expression pattern of Fgf10 in the duodenum at a late stage of development. The blue staining...
3. Discussion

Duodenal atresia is one of the more commonly treated congenital malformations seen by pediatric surgeons. Many have studied duodenal atresia with the aims of discovering its etiologic roots and improving its treatment. The scientific method and its tools of the day have been applied for more than a century. Today’s genetic tools are being applied to the study of duodenal atresia and its sequelae (namely, postoperative dysmotility, short bowel syndrome, and feeding difficulties).

Many authors have linked duodenal atresia to a genetic cause, but none have studied a specific gene’s role in embryology and its implications in the pathogenesis of duodenal atresia. We have demonstrated that Fgf10 is not only active during the development of the duodenum but that its role is also critical to normal development. The phenotype of our mice is extreme, as indicated by the devastation that the deletion of FGF10 wreaks on multiple organ systems. Because of the multiple anomalies, it is unlikely that human embryos suffering a total deletion of FGF10 would survive. A study by Rosano et al [18] has linked duodenal atresia (as well as other gastrointestinal tract malformations) to other birth defects, which suggests the pervasiveness of defects in development-regulating genes. We suggest that a minor defect in FGF10 could amplify during development to a larger constellation of genetic defects. If such a defect were to occur and this defect regulated functions outside the redundancy inherent in organogenesis, then FGF10 would be implicated in a much larger syndrome. This opens the door to studying the role of Fgf10 in novel therapeutics in the future.

Aside from the medical applications, this work on Fgf10 will have more immediate uses as a model. Current animal models used to study duodenal atresia all rely on some external intervention of the investigator. This introduces some degree of error into any study conducted on duodenal atresia. Early studies of intestinal atresia by Louw and
Barnard [2] involved the surgical ligation of mesenteric vessels in utero. Few of the study animals survived. Other models requiring some surgical intervention to create an atresia have used lambs [19] and chicks [20]. A nonsurgical model for duodenal atresia was created after studying esophageal atresia [21]. This model relies on the administration of adriamycin, which also does not replicate a normal gestational course. As the prominence of genetics continues to grow in medicine, a genetic model for human diseases (duodenal atresia in this case) becomes more crucial to the scientific pursuit of these diseases. This is the first report of a readily reproducible, genetically derived animal model for studying duodenal atresia. This model should prove useful in the study of this common surgical disease and improve our understanding of its pathogenesis and its implications.

References