Embryogenesis of the congenital anomalies of the kidney and the urinary tract

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Abstract
Ectopia of the initial ureter is the first ontogenic misstep that leads to many congenital anomalies of the kidney and urinary tract (CAKUT). The ectopia results in hypoplastic kidney, ectopia of the ureteral orifice, urinary outflow obstruction and/or reflux. Recent studies on several mutant mouse models verified that ectopic ureteral budding indeed occurs prior to the formation of CAKUT. Often, the genes involved in navigating the site of ureteral budding also regulate later ontogenic processes of the kidney and other urinary tract systems. These additional functions of the genes underline the wide spectrum of CAKUT, as the genes are expressed at multiple sites at multiple ontogenic stages, and regulate the morphogenesis of the many portions of the excretory system through their distinctive cellular functions.

Keywords: angiotensin; BMP4

Congenital anomalies of the kidney and urinary tract (CAKUT) are a family of diseases with a diverse anatomical spectrum, including kidney anomalies, e.g. multicystic dysplastic kidney, hypoplastic kidney, ureteropelvic junction obstruction, vesicoureteral reflux and megaureter [1]. These abnormalities are often present simultaneously, and take a familial pattern, with incomplete and variable penetrance, often producing different anatomical patterns [2]. It has been speculated, therefore, that these assorted structural anomalies share common pathogenic mechanisms and genetic causes.

Major investigative efforts have been made over the last decade to identify the specific gene(s) that are responsible for these anomalies, specifically PAX2 [3] and EYA1 [4]. To explain how such a wide anatomical spectrum of anomalies occurs in the kidney and urinary tract system, several theories have been formulated. These include: (i) physical stress on the kidney and the ureter as a result of a urinary outflow obstruction; (ii) physical stress as a result of dysfunction of the bladder or the vesicoureteral junction; (iii) ectopia of the initial budding of the ureter from the Wolffian duct; and (iv) a primary defect in the interaction at the cellular level between the metanephric mesenchyme and the ureteric bud.

A unique, but now popular, theory was derived from an extensive inspection of numerous specimens from human embryos and neonates. This theory stems from observed morphological correlations among the location of the ureteral orifice, the degree of renal hypo- and/or dysplasia and the abnormalities of the ureter [5]. The hypothesis proposes that these abnormalities are derived from a single common mechanism and are programmed at the time of initial budding of the ureter from the Wolffian duct. Note that in the developing embryo, the terminal segment of the Wolffian duct is absorbed into the cloaca to form the hemitrigone of the developing bladder. In this process, therefore, the initial budding site of the ureter normally will migrate and reach its final destination, i.e. the corner of the bladder trigone, to form the ureteral orifice in the bladder. Mackie and Stephens [5] postulated that when ureteral budding occurs at an ectopic site, the final site of the ureteral orifice will be ectopic as well, thereby often resulting in urinary outflow obstruction. Besides these, ectopic budding has the potential to produce anomalous kidney parenchyma as the bud from the ectopic site makes contact with poorly differentiated portions of the metanephric mesenchyme, which becomes the precursor for the later hypo- and/or dysplastic kidney. Therefore, the anomalies of multiple tissues constituting CAKUT (i.e. abnormal ureterovesical junction, dysmorphic kidney and, as discussed later, anomalous
ureter) can be derived from a single abnormal embryonic event (i.e. ectopia in the initial ureteral budding). Although the Mackie and Stephens’ hypothesis can explain comprehensively the ontogeny of CAKUT, the hypothesis has remained untested, because, even if ectopic budding occurs, it is impossible to verify it prior to the appearance of CAKUT, since the incidence of the latter is at best sporadic. In this regard, the recent discovery of genetic mouse models carrying a high incidence of CAKUT has, for the first time, made it possible to examine if ectopic budding indeed occurs in embryos. Of the two receptors for angiotensin II, angiotensin type 2 receptor is the one only recently identified [6] and its gene (Agtr2) is primarily an embryonic gene, i.e. in humans and animals, Agtr2 is actively transcribed at the onset of, and throughout, the embryonic development of the kidney and urinary tract system and mostly inactivated by the time of birth [7,8]. When Agtr2 was target inactivated by genetic engineering technology, it was noted that mutant mice, at the rate of 2–3%, have anomalies in the kidney and urinary tract system, which remarkably resemble those of human counterparts [9].

A study in human DNA samples indicated that the same angiotensin type 2 gene is involved in human CAKUT [9]. Thus, it was found that a minority of the general population carries a functionally significant mutation within the so-called lariat branch point of intron 1, which results in a significant alteration in the quality and quantity of the mRNA due to abnormal mRNA splicing. A pair of independent DNA studies on American and German populations of patients with CAKUT revealed a significant correlation between the incidence of CAKUT and this specific mutation [9].

Bone morphogenetic protein 4 (BMP4), a member of the transforming growth factor-β (TGF-β) superfamily of secretory signalling molecules, has been implicated in many aspects of embryonic development [10]. Detailed examination of the excretory system in heterozygous mutants (Bmp4+/−) revealed that some 50% of Bmp4+/− mice have anomalies, which closely mimic human CAKUT, including hypo-/dysplastic kidneys, hydroureter and a double collecting system [11]. It is of note that, similarly to human CAKUT, this mouse CAKUT involves the ectopic ureteral orifice, i.e. the location of the orifice being abnormally caudal to the normal site. Analyses of the mutant embryos showed that this ectopia of the ureteral orifice is a consequence of the ectopic ureteric budding from the Wolffian duct.

Studies in mutant animals point to the notion that ectopia in the initial budding is a very common first step leading to the formation of CAKUT. It is of note that the expression of the many regulatory molecules

![Fig. 1. Pluripotentiality of the single gene mutation underlies the wide spectrum of clinical anomalies involving the ureterovesical junction, the ureter and the kidney. The loss-of-function mutation of the single gene can produce multiple anomalies (1) due in part to its multiple biological actions on the morphogenesis of the three tissues of the excretory system, i.e. the ureterovesical junction, the ureter and the kidney. It is also (2) due to the multipotentiality of the initial ectopic budding to produce three clinical entities, i.e. ectopic ureteral orifice, anomalous ureter and hypo-/dysplastic kidney as postulated by Mackie and Stephens [5].]
for kidney and urinary tract morphogenesis is not limited to the site or the timing of initial ureteric budding, but instead continues throughout kidney development. It is possible, therefore, that some molecules which regulate the initial budding of the ureter also regulate the subsequent ontogenic processes of the kidney and urinary tract. Accordingly, some anomalies in CAKUT may be attributed to a derangement in these late ontogenic processes rather than the ectopia of the initial budding per se.

Indeed, an intense Bmp4 expression continues beyond the initial budding stage, and throughout the rest of intrauterine life at various sites of the excretory system, including loose stromal mesenchymal cells around the main trunk and the stalk of the branching ureter, and the epithelium of comma- and S-shaped bodies. Studies on heterozygous Bmp4 knockout embryos in vivo and cultured explants in vitro showed that BMP4 has multiple biological functions in the morphogenesis of the excretory system [12]. First, in addition to the inhibitory effect on the ureteric bud formation, BMP4 promotes the growth and elongation of ureteric buds once buds have formed. Secondly, BMP4 acts on the metanephric mesenchyme to prevent apoptosis, promotes growth of the stromal cell population and inhibits condensation of the mesenchymal cells around the ureteral bud. Furthermore, BMP4 can serve as a chemotactant for the periureteral mesenchymal cells and, in doing so, induces locally the smooth muscle layer of the ureter.

Likewise, Agtr2 also appears to have diverse regulatory roles since its expression continues throughout kidney development. It has indeed been shown in vivo and in vitro that activation of the Agtr2 receptor promotes timely apoptosis of the undifferentiated mesenchymal cells that surround the smooth muscle layer of the ureter, and a defect in this function of Agtr2 may lead to atresia of the ureter found in some Agtr2 null mutant mice by not permitting normal enlargement of the ureter and/or supplying vessel calibre [9]. In conjunction with the notion that many embryonic genes govern organogenesis at various stages and in various tissues, the diversity of CAKUT can be attributed to the fact that many of the genes involved in the formation of CAKUT are multifunctional, participating in multiple aspects of the ontogeny of the excretory system. Thus, a wide spectrum of anomalies can result from a mutation in the gene which can regulate not only the site of the ureteral orifice (via determination of the site of the initial ureteric bud), but also of the kidney and the ureter at later stages, each through a distinct mechanism (Figure 1).

References