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Primitive Ducts of Renal Dysplasia Induced by Culturing Ureteral Buds Denuded of Condensed Renal Mesenchyme

Abstract. *Primitive ducts, the histological hallmark of human renal dysplasia, were induced in chick embryos by culturing ureteral buds denuded of condensed metanephrogenic mesenchyme.*

Human renal dysplasia, a relatively common malformation, is diagnosed by noting "primitive ducts" in biopsy specimens. The primitive duct is lined by a tall epithelium and is surrounded by a collar of whorled fibromuscular cells (*1*). The duct has been believed to result from injury to the branches of the ureteral bud, but to our knowledge there is no evidence to support this.

In an attempt to clarify the morphogenesis of the primitive duct and thereby improve our understanding of the etiology of renal dysplasia, we studied the development of renal blastemas isolated from chick embryos. We found that (i) isolated renal blastemas grafted onto a chorioallantoic membrane in ovo develop histologically normal renal architecture; (ii) when isolated renal blastemas are cultured in vitro, the ureteral bud branches lose the apposed condensed metanephrogenic mesenchyme; (iii) ureteral buds denuded of condensed metanephrogenic mesenchyme by preliminary tissue culture develop further in ovo as chorioallantoic grafts and form primitive ducts; and (iv) primitive ducts induced in the chick resemble those noted in human renal dysplasia. These findings suggest that the primitive duct of renal dysplasia originates from branches of the ureteral bud that develop without condensed metanephrogenic mesenchyme.

Normal nephrogenesis in the human embryo requires interactions in the renal blastema (*2*), which consists of the ureteral bud and the metanephrogenic mesenchyme. The ureteral bud branches as an arcade and develops into the ureter, pelvis, calyces, and collecting ducts. The metanephrogenic mesenchyme apposes the ampullae of the ureteral bud branches and develops into renal tubules and glomeruli.

Although approximately 10 percent of children are born with potentially significant malformations of the urinary tract (*3*), the morphogenesis of most renal malformations is unknown. Current views are that renal dysplasia arises in utero as the result of abnormal interaction between the ureteral bud and

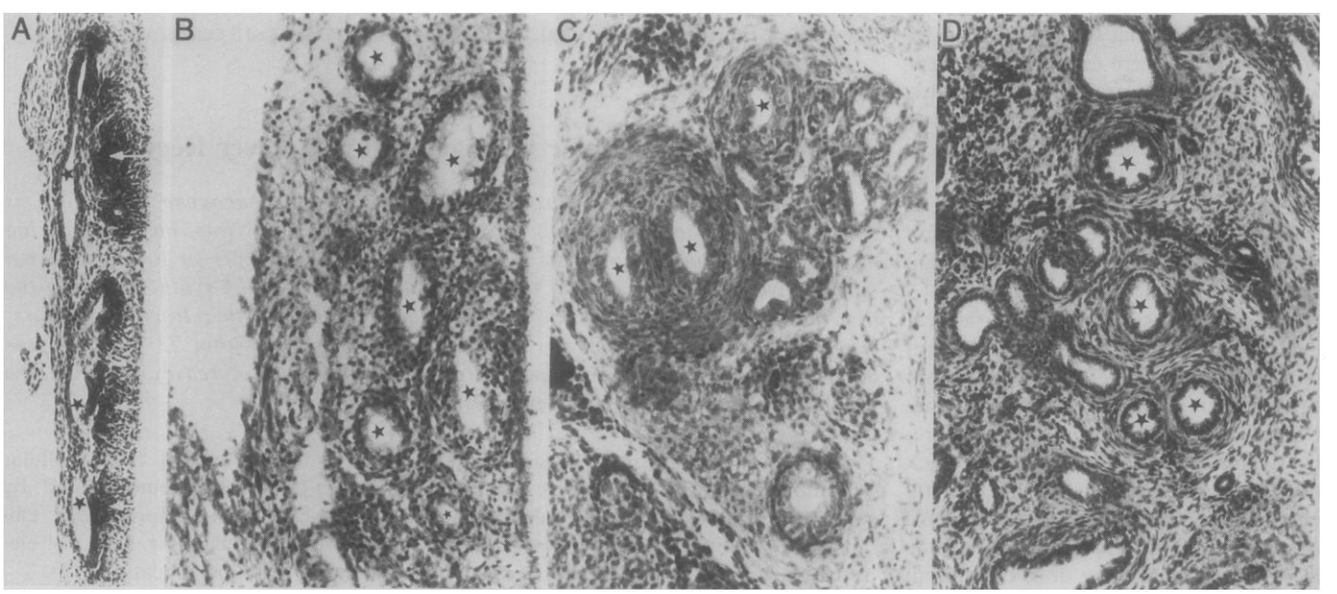

Fig. 1. Induction of primitive ducts. (A) Longitudinal section of renal blastema microdissected from chick embryo after 8 days of incubation. Visible are segmental branches of the ureteral bud (★) and condensed metanephrogenic mesenchyme (arrow) (×160). (B) Renal blastema microdissected from chick embryo after 7 days of incubation and then cultured in vitro for 3 days. Ureteral bud branches (★) are numerous, but the metanephrogenic mesenchyme is no longer condensed or apparent. Renal tubules do not develop in vitro (×160). (C) Renal blastema microdissected from chick embryo after 8 days of incubation, then cultured in vitro for 7 days to provide branched ureteral buds without condensed metanephrogenic mesenchyme, and finally further cultured in ovo as a graft that developed into tissue composed primarily of primitive ducts (★). The ducts are lined by tall epithelium and are surrounded by whorled mesenchymal cells (×160). (D) Nephrectomy specimen in newborn with prune belly syndrome. Primitive ducts (★) typical of renal dysplasia are surrounded by whorled fibromuscular cells and resemble those induced in the chick embryos (×100).

its metanephrogenic mesenchyme (*4*), obstruction of urine drainage (*1*), or interference with the development of the ampullae of the ureteral bud branches (*5*). These possibilities require evaluation. Experimental ligation of the fetal or embryonic ureter obstructs urine drainage but is not always associated with the later development of dysplasia (*1, 6–8*). These discrepant results suggest that factors other than simple obstruction must be considered in the genesis of dysplasia.

Renal blastemas were microdissected from chick embryos in days 7 to 8 of incubation. At this stage, the renal blastema is an elongate, branched ureteral bud apposed by condensed metanephrogenic mesenchyme (Fig. 1A); nephrons are not yet present. Seventeen blastemas were cultured on the chorioallantoic membrane as an in ovo graft for up to 10 days. These normal blastemas developed a normal renal architecture (13 specimens) or mild hydronephrosis (four specimens). Twenty-six other blastemas were placed in tissue culture (*9*) for up to 7 days. These explants displayed branching of the ureteral bud, but the metanephrogenic mesenchyme did not remain condensed (Fig. 1B). Finally, 33 renal blastemas deprived of condensed metanephrogenic mesenchyme by preliminary tissue culture were further cultured as chorioallantoic grafts. This procedure resulted in kidneys that displayed normal architecture, normal architecture with a few primitive ducts, many primitive ducts with few renal tubules, or primitive ducts only (no renal tubules) (Table 1). Primitive ducts were especially frequent when blastemas were cultured in vitro for at least 4 days ($P < .005$, chi-square test) (Fig. 1C). Both the primitive ducts induced in the chick embryos and those present in 11 human dysplastic kidneys (Fig. 1D) demonstrated tall epithelium surrounded by whorled mesenchymal cells.

Our results suggest that primitive ducts originate from branches of the ureteral bud that develop without condensed metanephrogenic mesenchyme. Cartilage, another feature of human renal dysplasia, was not seen in this chick model. It may be that the chick metanephrogenic mesenchyme is not capable of metaplastic differentiation or that the brief period of grafting does not permit mesodermal expression into cartilage. That chick renal blastemas explanted in tissue culture did not further differentiate may be accounted for by the inability of new tubules to be fostered in vitro.

There has been little investigative effort to clarify the morphogenesis of genitourinary malformations. Attempts to induce renal dysplasia experimentally have been directed at obstructing the urine drainage of fetal (*6, 7*) or neonatal (*1*) kidneys. These attempts have not been uniformly successful, perhaps because of the timing of the experimental manipulations (*10*). The most severe cases of dysplasia are believed to develop from insults that affect the kidney before nephrogenesis (*11*). The model described here provides a unique means of interfering with the development of the embryonic kidney before the appearance of renal tubules. Obstructing the urine drainage of the embryonic kidney in this model does not cause dysplasia. (*8*).

Table 1. Histological development of renal blastemas cultured in vitro and in ovo. Values are numbers of grafts showing the indicated architectures.

Days in tissue culture	Architecture of graft*			
	Normal	Some primitive ducts	Many primitive ducts	Primitive ducts only
0	17	0	0	0
1 to 3	7	5	1	1
4 to 7	2	4	8	5

*Includes specimens that exhibited dilation of the ureter or tubules; these changes may be interpreted as hydronephrosis.

In conclusion, it appears that perturbing the condensed metanephrogenic mesenchyme of the embryonic kidney leads to changes consistent with renal dysplasia. These experiments support the notion that congenital renal malformations arise from faulty nephrogenesis. They also provide a basis for further manipulation of embryonic tissue to simulate congenital urologic malformations. Such simulations could provide a better understanding of the morphogenesis of congenital malformations.

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Expression of a Cellular Oncogene During Liver Regeneration

Abstract. *The number of transcripts of the cellular oncogene* ras, *which is homologous to the transforming gene of Harvey sarcoma virus, increases during liver regeneration in rats. The increase in these transcripts in liver polysomal polyadenylated RNA occurs at the time of activation of DNA synthesis during the regenerative process induced by partial hepatectomy or carbon tetrachloride injury. The number of* ras *transcripts returns to basal levels within 72 hours. These observations show that transcription of a cellular oncogene increases in a regulated way in a nonneoplastic growth process.*

Transforming retroviruses originate by recombination of type C RNA viruses with cellular sequences of vertebrate hosts (*1, 2*). Studies with temperature-sensitive and deletion mutants indicate that the cellular sequences incorporated into these viruses are necessary for virus-induced cell transformation (*3*) and for rapid production of tumors in vivo. So far, more than a dozen different viral transforming sequences (v-*onc*) have been identified, each with a cellular counterpart (c-*onc*) (*1, 2, 4, 5*). The oncogenicity of several of these cellular sequences has been demonstrated by their capacity to transform cells efficiently when linked to viral control elements (long terminal repeats) (*6, 7*).

Because cellular oncogenes are highly conserved and transcribed in uninfected normal cells, they might play a role in nonneoplastic growth processes and in nonviral neoplastic growth (*1, 2, 4, 5, 8*). We now present data on the expression of the Harvey sarcoma virus *ras* gene during liver regeneration induced by par-