Signal Transduction in Renal Development

Cell turnover in normal and abnormal kidney development

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Abstract
As metanephric mesenchyme converts into nephrons, the first step is aggregation into a ‘condensate’. Precursors inside this structure are proliferative and have a low rate of apoptosis, accompanied by expression of PAX-2 and BCL-2 survival molecules; conversely, cells at the borders of the structure have a high rate of apoptosis, probably a normal mechanism to regulate the number of cells in each nephron. Ureteric bud collecting duct survival and mitosis may be determined partly by renal mesenchymal secreted molecules such as hepatocyte growth factor (HGF) and glial cell line-derived neurotrophic factor. Human kidney malformations often occur with lower urinary tract obstruction, e.g. cystic dysplastic kidneys caused by urethral valves. Deregulation of cell turnover occurs in these organs, with enhanced proliferation in cystic epithelium, accompanied by PAX-2, BCL-2 and HGF receptor expression, and apoptosis in surrounding mesenchyme, which transdifferentiates under the influence of transforming growth factor-β1 into smooth muscle instead of forming nephrons. Similar abnormalities of cell turnover and gene expression can be generated by experimental fetal urinary flow impairment. Finally, renal mesenchymal apoptosis, associated with renal hypoplasia, can be induced experimentally by maternal low protein diet.

Keywords: apoptosis; epithelium; malformation; mesenchyme; proliferation

Introduction
The precursor of the adult mammalian kidney is called the metanephros and it appears at 5 weeks of human gestation, equivalent to embryonic day 11 in mice and day 12 in rats. At this stage, the organ consists of ureteric bud epithelium which becomes enveloped by renal mesenchyme: these tissues form collecting ducts and nephron tubules, respectively. As well as cell differentiation and morphogenesis, normal kidney development involves a strict control of proliferation and programmed cell death, mediated by apoptosis.[1,2].

Metanephric mesenchyme is programmed to die unless ‘rescued’ by poorly defined factors from the ureteric bud.[3,4]. As it differentiates into nephron epithelia, its cells aggregate. Precursors in the centre of this cell mass have a high index of proliferation and a low index of apoptosis, and they express PAX-2, a paired-box transcription factor, and BCL-2, a cell survival molecule.[5]. Conversely, limited apoptosis occurs in cells on the borders of mesenchymal aggregates[1,2]; this is most probably a normal mechanism to regulate the number of cells in each nephron.

Mice with two mutated pax-2 alleles never initiate metanephric development, whereas mice with heterozygous mutations develop small kidneys with too few nephrons: the latter animals phenotypically resemble humans with the renal–coloboma syndrome, and these individuals also have PAX-2 mutations.[6]. Recent experiments in vivo[7] and in vitro[8] demonstrate that PAX-2 is anti-apoptotic. Mice with null mutations of bcl-2 initiate metanephric development but these organs undergo fulminant apoptosis resulting in renal hypoplasia.[9]. Furthermore, specific growth factors modulate renal mesenchymal survival, with death reduced by bone morphogenetic protein-7, epidermal growth factor and fibroblast growth factor-2,[1,3,4,10], but enhanced by tumour necrosis factor-α (TNF-α)[11].

Epithelia in the branching tips of the ureteric bud are highly proliferative[5]; their mitosis and survival are determined by signals secreted by adjacent mesenchyme such as hepatocyte growth factor (HGF)[12] and glial cell line-derived neurotrophic factor.[13]. The hilum of the metanephros is another ‘hot-spot’ for apoptosis, and this may be related to the growth of blood vessel precursors. As development proceeds, both mitosis and apoptosis decrease[1,2,5], as does the expression of nephrogenic survival factors.

Cell turnover in human kidney malformations
When the complex events described above go wrong, diverse kidney human and urinary tract malformations
are generated: these include agenesis (absent kidney), dysplasia (abnormal differentiation, often with cysts) and hypoplasia (too few nephrons). These disorders account for the great majority of young children in chronic renal failure. Major deregulations of cell turnover have been documented in human dysplastic kidneys, organs comprised of poorly branched tubules surrounded by incompletely differentiated and metaplastic stromal cells which express α smooth muscle actin (αSMA).

First, enhanced proliferation occurs in dysplastic epithelia, accompanied by PAX-2, BCL-2 and HGF receptor expression [5,14,15]; indeed, dysplastic tubules can grow to form massive cysts which distend the abdomen. In the same organs, increased apoptosis can be documented in surrounding dysplastic stroma where PAX-2 and BCL-2 expression are low [2,5]; this results in a loss of potential nephron precursors and may account for the spontaneous involution which can occur of these bizarre, enlarged kidneys. TNF-α is also up-regulated in these organs, and might contribute to the dysplastic phenotype by enhancing apoptosis and perturbing normal branching [11,16]. Recently, Yang et al. [15] reported a further aberration of cell biology in human dysplastic kidneys: under the influence of up-regulated transforming growth factor-β1, dysplastic epithelia down-regulate proliferation, PAX-2 and BCL-2, and form metaplastic smooth muscle with up-regulation of αSMA and fibronectin. This causes a 'loss' of epithelia which might otherwise branch to form collecting ducts.

Human kidney malformations often occur in association with lower urinary tract obstruction, e.g. multicystic dysplastic kidneys attached to atretic ureters, or cystic dysplastic kidneys associated with posterior urethral valves. It is notable that experimental ureteric obstruction in fetal sheep can generate abnormalities of cell turnover and gene expression similar to those documented in human dysplastic kidneys [17,18].

Metanephric apoptosis and maternal diet

Certain common adult diseases may originate by ‘embryonic programming’. For example, individuals born to mothers with poor diets are prone to hypertension [19]. Investigators are exploring the biological bases of programming. Rats born to mothers on low protein diets have fewer nephrons than normal and become hypertensive [20], experiments complementing human studies reporting nephron deficits in infants with intrauterine growth retardation [21]. Furthermore, congenital nephron deficits interact with environmental influences, such as hyperglycaemia, and with genetic factors to generate glomerulosclerosis [22].

We hypothesized that low protein diets ‘programme’ the metanephros by altering cell turnover so that there are fewer precursors available to differentiate into nephrons. We exposed pregnant Wistar rats to diets varying in protein (casein) but containing similar calories and vitamins: 18% protein for the control group, and 9 or 6% protein for the moderate and severe protein restriction groups, respectively [23]. At 2 weeks after birth, by which time nephron formation had ended, numbers of glomeruli per kidney were significantly reduced in offspring in both low protein groups. Next, we examined metanephroi at embryonic day 13, when renal mesenchyme is aggregating, but nephrons have yet to form, and when the ureteric bud has branched once or twice. At this stage, programmed cell death was almost confined to the mesenchymal compartment, being very rare in the ureteric bud lineage. Apoptotic indices (nuclei/area), as assessed by in situ end labelling and propidium iodide-stained pyknotic nuclei, were significantly increased in embryos of mothers fed low protein diets. We currently are attempting to elucidate the molecular mechanisms which mediate this striking effect.

Conclusions

These results indicate that: (i) a finely regulated degree of apoptosis occurs in normal metanephric development; (ii) a simple physical insult, such as ureteric urinary flow impairment, results in complex aberrations of metanephric biology including deregulated proliferation and cell death; and (iii) nephron deficits, accompanied by enhanced metanephric apoptosis, are induced by extra-embryonic changes in environment, such as altered maternal diet.

References


