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A spatially-averaged mathematical model of kidney branching morphogenesis

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HIGHLIGHTS

- We have developed a spatially-averaged mathematical model of kidney morphogenesis which accounts for interactions between epithelial tip and mesenchymal cap cell populations.
- Our model can be used to estimate the number of branch generations at different stages during kidney development. It predicts that developing kidneys respond differently to loss of cap mesenchyme versus ureteric tip cells, and that augmenting the growth rate of mesenchymal cells will more effectively increase branch number than augmenting the growth rate of the tip cells. Additionally, the model predicts that cessation of kidney branching requires an active trigger at the time of birth.

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ABSTRACT

Kidney development is initiated by the outgrowth of an epithelial ureteric bud into a population of mesenchymal cells. Reciprocal morphogenetic responses between these two populations generate a highly branched epithelial ureteric tree with the mesenchyme differentiating into nephrons, the functional units of the kidney. While we understand some of the mechanisms involved, current knowledge fails to explain the variability of organ sizes and nephron endowment in mice and humans. Here we present a spatially-averaged mathematical model of kidney morphogenesis in which the growth of the two key populations is described by a system of time-dependant ordinary differential equations. We assume that branching is symmetric and is invoked when the number of epithelial cells per tip reaches a threshold value. This process continues until the number of mesenchymal cells falls below a critical value that triggers cessation of branching. The mathematical model and its predictions are validated against experimentally quantified C57Bl6 mouse embryonic kidneys. Numerical simulations are performed to determine how the final number of branches changes as key system parameters are varied (such as the growth rate of tip cells, mesenchyme cells, or component cell population exit rate). Our results predict that the developing kidney responds differently to loss of cap and tip cells. They also indicate that the final number of kidney branches is less sensitive to changes in the growth rate of the ureteric tip cells than to changes in the growth rate of the mesenchymal cells. By inference, increasing the growth rate of mesenchymal cells should maximise branch number. Our model also provides a framework for predicting the branching outcome when ureteric tip or mesenchyme cells change behaviour in response to different genetic or environmental developmental stresses.

1. Introduction

Mammalian kidneys are vital organs that filter waste products from the blood while also regulating blood volume, blood pressure and acid balance. These functions are achieved via specialised filtration/reabsorption structures called nephrons. While all

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nephrons are formed during embryonic development, there is substantial variability in final nephron number with this ranging from 300,000 to 1.8 million nephrons per kidney in humans (Bertram et al., 2011). The functional consequences of this variability are now becoming apparent, with evidence of an inverse relationship between nephron number and renal function during adult life (Moritz et al., 2003). Our understanding of what regulates final nephron number remains incomplete. Overt abnormalities of the kidneys, including hypoplasia/dysplasia, are observed in as many as 1 in 200 newborns (Weber et al., 2006), and a large number of genes have been implicated. There is additional evidence for an impact of a variety of *in utero* environmental stressors on nephron number, including protein deprivation, drug and ethanol consumption and altered glucocorticoid levels (Moritz et al., 2003). Premature birth appears to accelerate the cessation of nephron formation, resulting in reduced nephron number in such individuals (Faa et al., 2010).

The mammalian kidney is formed from several key progenitor cell types. An initial epithelial outgrowth, the ureteric bud (UB), extends from the nephric duct of the embryo in response to a source of chemoattractive ligands in an adjacent mesenchymal population, the metanephric mesenchyme, E10.5 in mouse, week 5 in humans (Little and McMahon, 2012). Once these two populations meet, the ureteric bud commences a process of mainly dichotomous branching events to form a characteristic 'ureteric tree' that constitutes the exit path for urine produced by the nephrons. Conversely, the mesenchyme aggregates around the tips of the branching ureteric epithelium to form the cap mesenchyme. The cap mesenchyme surrounding a given UB tip is referred to as a nephrogenic niche and this niche remains the location of subsequent branching events as well as the location of a second morphogenetic event, the differentiation of a portion of cap mesenchyme cells into non-branching epithelial nephrons. Subsequent UB branching is supported by the presence of the mesenchyme whereas survival of the mesenchyme and its differentiation into nephrons is regulated, at least in part, by signals secreted by the epithelial UB tip cells.

While the processes driving kidney morphogenesis are complex, the role of a number of key growth factors is known. Glial derived neurotrophic factor (Gdnf) produced by the mesenchyme, is the initial, chemoattractive signal and the morphogen that subsequently drives continued branching of the ureteric tree. In response to Gdnf signalling, the UB cells upregulate the Ret receptor which in turn upregulates Wnt11 (Costantini and Kopan, 2010). Wnt11 production by the UB cells is thought to feed forward to increase Gdnf production within the mesenchyme (Majumdar et al., 2003). This central pathway can, however, be bypassed via FGF10 signalling, explaining the robustness of the process of initial kidney formation (Costantini and Kopan, 2010). The fate of the mesenchyme is a balance of two processes: differentiation into nephrons and proliferation. The production of Wnt9b within the UB drives differentiation of mesenchyme into nephrons (Carroll et al., 2005), but may also support its survival (Karner et al., 2011), whereas the production of Fgf9 in the UB upregulates *Fgf9* and *Fgf20* within the mesenchyme ensuring ongoing proliferation of that cellular compartment (Barak et al., 2012). This crosstalk between the two cell types is assumed to regulate their rates of proliferation as well as their respective transformation into other cell types.

A number of mammalian organs form via branching morphogenesis of an epithelial outgrowth. While organs such as the lung have a more defined primary branching program (Metzger et al., 2008), synchronous and asynchronous dichotomous branching drive the bulk of lung development in a manner similar to other organs including the kidney (Short et al., 2013). In both cases, signals exist within the surrounding mesenchyme and that mesenchyme has been shown to play an instructive role, although not necessarily on organ shape but on final differentiation (Taylor et al., 2009). In each case, the distal-most portion of the branching epithelial tree is also important for organ function, with differentiation of the epithelial tips forming specialised structures. In the case of the lung, the epithelial tips form the alveoli whereas in the mammary gland they form the milk-producing acini. In contrast, branching of the ureteric tree of the kidney results in more than an arborized tree with specialised tips: branching is coupled with the induction of a differentiation event within the mesenchyme to form a second tubular structure, the nephron, which fuses with the tips. In this way, the reciprocal interaction of the mesenchyme and ureteric tip populations is a unique inter-relationship in mammalian organogenesis. However, the fact that the ureteric tree will form a dichotomously branched structure in vitro, in the absence of the mesenchyme (Nigam and Shah, 2009), suggests a cell-autonomous branching program.

During kidney development, we have little understanding of what initiates any given branching event, how perturbation of proliferation within the ureteric or mesenchymal compartments will affect this branching or how it is influenced by ongoing nephron formation. One may predict that if survival or proliferation of either the mesenchyme or the epithelium were suppressed, this might retard, or even terminate, development. Certainly, a global switch for the mesenchyme to differentiate rather than proliferate (self-renew) completely represses subsequent branching (Self et al., 2006). Conversely, some genetic examples of hypoplasia, with an overall reduction in ureteric branching, show evidence for a reduction in ureteric epithelial proliferation (Cain and Rosenblum, 2011). However, what causes the process to end under normal circumstances is not known. Branching does slow across developmental time (Cebrian et al., 2004), indicating that the relationship between these populations changes with time. The final nephrons form in the immediate postnatal period, arguably in both mouse (Rumballe et al., 2011) and humans (Faa et al., 2010). Whether this represents the inevitable consequence of the relationship between the two progenitor populations or the imposition of another signal is not known, but will be important in understanding how we might ameliorate challenges such as those associated with premature birth.

There are very few mathematical models of mammalian organogenesis, and most of these focus on lung morphogenesis. Metzger et al. (2008) documented a stereotypic process of branching in the mammalian lung, proposing a small subset of branching routines that reproducibly play out at distinct times and positions. A mathematical model based on diffusion-limited growth has been proposed to describe the regulation of branching during lung morphogenesis (Hartman and Miura, 2006). In an alternative approach, the mesenchymal and epithelial tissues were modelled as distinct viscous fluids, separated by a moving boundary whose shape changes were assumed to mimic branching (Lubkin and Murray, 1995). In the kidney, Hirashima et al. (2009) used a cellular Potts model to investigate how the balance between cell proliferation and chemotactic cell movement could influence the pattern of ureteric branching in vitro. In this model, branching was assumed to be driven solely by a signal from the adjacent mesenchyme, however isolated ureteric epithelium has an intrinsic capacity to branch (Qiao et al., 1999). More recently, initiation of kidney branching has been simulated using a ligand-receptor based Turing model that represents the known Gdnf – Ret – Wnt11 feed forward loop (Menshykau and Iber, 2013) and this has been refined into a laboratory based model based on organ culture of kidneys (Adivarahan et al. (2013)). Organ culture, while useful for simple studies of branching, does not recapitulate normal branching mechanics (Short et al., 2010). None of these studies have used data from in vivo kidneys, across development and in three