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Cap mesenchyme cell swarming during kidney development is influenced by attraction, repulsion, and adhesion to the ureteric tip

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ABSTRACT

Morphogenesis of the mammalian kidney requires reciprocal interactions between two cellular domains at the periphery of the developing organ: the tips of the epithelial ureteric tree and adjacent regions of cap mesenchyme. While the presence of the cap mesenchyme is essential for ureteric branching, how it is specifically maintained at the tips is unclear. Using *ex vivo* timelapse imaging we show that cells of the cap mesenchyme are highly motile. Individual cap mesenchyme cells move within and between cap domains. They also attach and detach from the ureteric tip across time. Timelapse tracks collected for >800 cells showed evidence that this movement was largely stochastic, with cell autonomous migration influenced by opposing attractive, repulsive and cell adhesion cues. The resulting swarming behaviour maintains a distinct cap mesenchyme domain while facilitating dynamic remodelling in response to underlying changes in the tip.

1. Introduction

Mammalian kidneys function to filter blood and regulate fluid homoeostasis in the body through thousands to millions of specialised filtration units called nephrons (Bertram et al., 2011; Merlet-Benichou et al., 1999). Filtrate from this multitude of nephrons is channelled to the bladder through a branched collecting duct system also known as the ureteric tree. The ureteric tree starts as an epithelial outgrowth from the posterior end of the Wolffian duct, which elongates and branches as it grows into the adjacent metanephric mesenchyme (Little and McMahon, 2012). Branching is driven by reciprocal interactions between the tips of the ureteric tree (ureteric tips, UT) and the cap mesenchyme (CM) that surrounds them (Costantini and Kopan, 2010). Factors produced by the CM, including GDNF and FGFs, stimulate proliferation and branching in the underlying tip epithelium while tip-produced factors, including WNT9B, maintain CM identity and also trigger CM differentiation to form nephrons (Carroll et al., 2005; Karner et al., 2011; Kopan et al., 2014). Each tip-cap domain, together with the surrounding stroma and vasculature, forms a nephrogenic niche. During branching, the mesenchymal population surrounding a given tip must self-renew to provide an ongoing 'cap' for the daughter tips. While lineage tracing definitively shows that a portion of CM cells in any niche 'exit' when induced to commit to nephron formation, it is the balance between nephron commitment and self-renewal that ensures that each niche is maintained to drive subsequent branching and hence ongoing nephron formation. Across development there is a steady reduction in niche size and number of cap and tip cells per niche, however the spatial arrangement of all niche components is maintained. Hence, the CM is only present out at the very periphery of the expanding organ and we do not currently understand how the CM domain is confined to this specific peripheral tip-associated location.

Morphogenesis involves cell movement. Cell movement within the tips of the ureteric epithelium has been documented using live imaging of flattened explant cultures (Chi et al., 2009; Packard et al., 2013; Riccio et al., 2016; Shakya et al., 2005; Watanabe and Costantini, 2004). However, little attention has been paid to cell movement within the CM and this cellular domain is currently described as if it were a static environment. Indeed, previous studies suggest spatially distinct cellular subdomains within the

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CM based upon differential gene expression, with this proposed to reflect a progressive commitment of cells within each subdomain to a differentiated state (Brown et al., 2013; Mugford et al., 2009). Furthermore, recent papers suggest that exposure to stromal signals around the periphery of the CM domain sensitises CM cells to differentiation (Das et al., 2013; Fetting et al., 2014; Mao et al., 2015). In a static environment, these results imply that peripheral cells are primed to differentiate while those closer to the tip are more likely to self-renew. Likewise, continued association of CM and UT could result from stable cell adhesion between these two domains. We and others recently published live imaging data of limited temporal and spatial resolution, which showed evidence for CM cell movement (Kanda et al., 2014; Lindstrom et al., 2015; Wainwright et al., 2015). The presence of cell motility within the CM raises the question of how a motile population maintains the form and appearance of a coherent domain.

In this study, we have quantitatively analysed patterns of cell movement for >800 individual cells in the cap mesenchyme using high resolution live imaging of kidney explants from transgenic reporter mice. Extensive cell motility was observed across an 18 h period with cells undergoing a number of previously undescribed behaviours. Cap cells oscillated between periods of 'free movement' and 'attachment' to the adjacent tip. Individual CM cells also dispersed within the niche, interacting with both tip and stromal environments over time and even crossing intervening stromal regions to join another niche. Observations of cell behaviour before and after tip attachment or cell division showed no correlation with changes in cell location or subsequent cell movement that may have indicated a differentiation event in this time window. Mathematical analyses of changes in cell position across time, compared with random movement, showed evidence for three contradictory forces influencing cell movement; cell adhesion to the tip, cell repulsion from the tip and attraction back to the tip. The balance of these competing forces combines to maintain the form of the CM domain and its relative position as the tip domains grow, split, and reposition during branching morphogenesis.

2. Results

2.1. The cap mesenchyme is motile during kidney morphogenesis

Kidney explant organ cultures were used to investigate CM cell motility (Costantini et al., 2011). This *ex vivo* culture method has been extensively used to investigate branching morphogenesis (Watanabe and Costantini, 2004), nephron patterning (Lindstrom et al., 2014) and tip cell fate in the developing kidney (Chi et al., 2009; Riccio et al., 2016; Shakya et al., 2005). Importantly, the CM domains in explanted kidneys appropriately associate with tip ends, continue to drive branching morphogenesis, and nephron induction and patterning occurs in an appropriate manner (Lindstrom et al., 2014). *Ex vivo* kidney explants from a CM-specific GFP reporter line (Six2-TGC$^{tg/+}$) (Kobayashi et al., 2008) were used for initial timelapse experiments. Low magnification imaging (10x) across 18 h revealed extensive and constant cell movement. Cells migrated within CM domains but also crossed freely between neighbouring domains (Fig. 1A-G, See also Supplementary Movie 1). Heterogeneity in cell speed and distance travelled was clearly apparent. Definitive assessment of CM cell behaviour during kidney development in vivo is not feasible with current approaches. However, analysis of SIX2^{+} cells in fixed 15.5 days *post coitum* (dpc) embryonic kidneys revealed that numerous individual cap

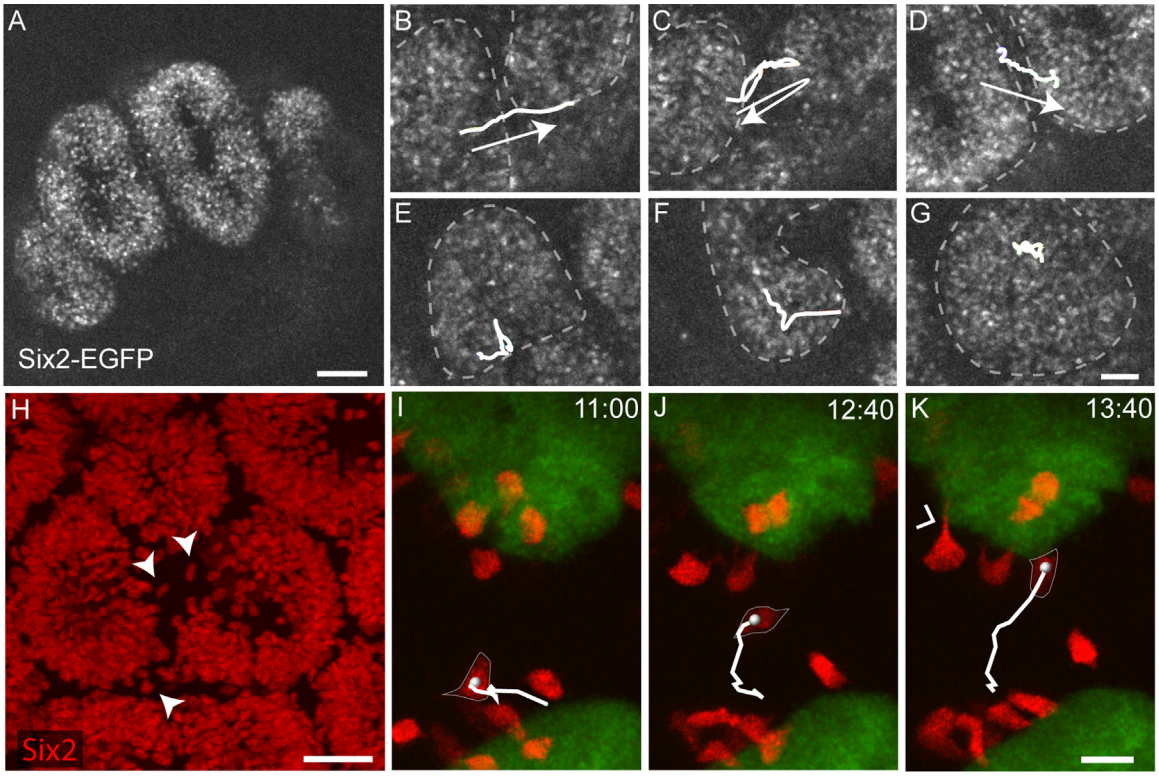

Fig. 1. SIX2+ cells migrate within and between CM domains. **A**) 10x confocal image of CM cells marked with nuclear EGFP (white. Scale bar 100 μm. **B-G**) Regions from A. White tracks indicate movement of cells between (B-D) or within (E-G) CM domains across time. Arrows indicate direction of movement. Scale bar for B-G (shown in G) 50 μm. **H)** Isolated SIX2+ cells are seen between domains in fixed 15.5dpc kidneys stained with SIX2 antibody (red). Scale bar 30 μm. **I-K)** A single CM cell migrates from one *Hoxb7-EGFP* (green) tip to another. Cell is marked with a sphere and outlined in white; track indicates movement in previous time frames. Time is indicated in hh:mm format in the top right corner. Open arrowhead illustrates a slender process extending from one labelled CM cell to touch the adjacent tip. Scale bar for I-K (shown in K) 20 μm.