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The regulation of growth and metabolism of kidney stem cells with regional specificity using extracellular matrix derived from kidney

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ABSTRACT

Native extracellular matrix (ECM) that is secreted and maintained by resident cells is of great interest for cell culture and cell delivery. We hypothesized that specialized bioengineered niches for stem cells can be established using ECM-derived scaffolding materials. Kidney was selected as a model system because of the high regional diversification of renal tissue matrix. By preparing the ECM from three specialized regions of the kidney (cortex, medulla, and papilla; whole kidney, heart, and bladder as controls) in three forms: (*i*) intact sheets of decellularized ECM, (*ii*) ECM hydrogels, and (*iii*) solubilized ECM, we investigated how the structure and composition of ECM affect the function of kidney stem cells (with mesenchymal stem cells, MSCs, as controls). All three forms of the ECM regulated KSC function, with differential structural and compositional effects. KSCs cultured on papilla ECM consistently displayed lower proliferation, higher metabolic activity, and differences in cell morphology, alignment, and structure formation as compared to KSCs on cortex and medulla ECM, effects not observed in corresponding MSC cultures. These data suggest that tissue- and region-specific ECM can provide an effective substrate for *in vitro* studies of therapeutic stem cells.

1. Introduction

The extracellular matrix (ECM), the native scaffolding material secreted and maintained by residents cells, provides an ideal microenvironment for cells, with tissue-specific physical and molecular cues mediating cell proliferation, differentiation, gene expression, migration, orientation, and assembly [1]. Functional and structural components within the ECM contribute to the extracellular environment specific to each tissue and organ [2–4]. The complexity of the ECM has proven difficult to recapitulate in its entirety, with attempts often limited to mimicking only ECM structure using synthetic biomaterials [5] or mimicking composition by adding purified ECM components [6]. And although they may offer structural mimics, synthetic biomaterials can potentially generate cytotoxic degradation by-products at the site of implantation, leading to poor wound healing or an inflammatory environment [7].

An alternative to synthetic biomaterials is to directly isolate native ECM from the tissue of interest via the removal of cells and cellular remnants. ECM scaffolds have been derived from a variety of soft tissues such as the small intestine, bladder, heart, liver, and lung [1,8,9]. ECM-derived biomaterials can be processed into scaffolds with appropriate compositions and structures for cell cultivation and tissue engineering. Furthermore, ECM scaffolds gradually degrade while promoting tissue remodeling at the site of implantation. Due to their biocompatibility and their ability to modulate the host tissue response, ECM scaffolds have shown promise for tissue engineering and regenerative medicine applications [1,8,9].

ECM-based scaffolds can also be used to regulate the differentiation and maintenance of stem cells and their differentiated progeny. Stem cells normally reside within a unique and highly regulated ECM, which contributes to the stem cell niche [10–14]. Historically, complex tissues such as heart and lung have been decellularized to obtain native-ECM scaffolds without particular regard for any specific region of the organ or preservation of

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potential stem cell niches [15,16]. Previous studies have shown that cells native to a particular region of the organ (e.g., vascular endothelium, liver sinusoidal cells) display ECM recognition and specificity [2–4]. If this site-specific recognition could be extended to stem cells, the choice of matrix would become an important consideration. However, there has been no study investigating differences in ECM derived from specific regions of an organ and how these regional differences could be preserved and harnessed for the *in vitro* culture of resident stem cell populations.

The kidney is a suitable organ for studying effects of regional ECM on the resident stem cell population. A cross-sectional view of the kidney reveals three distinct regions: cortex, medulla, and papilla (Fig. 1A), with each region displaying its unique structure, function, and composition, and residing in environments with very different osmolalities and oxygen tensions.

The cortex contains renal corpuscles, associated convoluted and straight tubules, collecting tubules and ducts, as well as an extensive vascular network. The medulla is arranged into pyramids, and characterized by straight tubules, collecting ducts, and the vasa recta, a specialized capillary system involved in the concentration of urine. At the apex of each medullary pyramid, where the collecting ducts converge and empty into the renal calyx, is the papilla. Recent studies have shown that the renal papillae contain a putative population of adult stem cells that remains quiescent after development is complete and that is mobilized after injury [10,12,17]. This stem cell population has been isolated in mice and expanded in culture, making the kidney an excellent model to study interactions between a native stem cell population and the matrix derived from distinct regions within the organ.

The present study describes a method to derive region-specific ECM biomaterials (ECM sheets, ECM hydrogels, and solubilized ECM) for stem cell culture from the three regions of the kidney (cortex, medulla, papilla). Our objective was to determine if there were region-specific effects of kidney ECM on the growth and metabolism of kidney stem cells, how these effects depend on the preservation of ECM structure versus composition alone, and if these effects extend to exogenous (non-kidney) stem cells, such as mesenchymal stem cells (MSCs).

2. Materials and methods

2.1. Decellularization

Porcine bladders, hearts, and kidneys were procured from Yorkshire pigs (65–70 kg) immediately following euthanasia, excess tissue was trimmed, and the blood and debris removed with water. The organs were stored at −80 °C for at least 24 h, thawed and then sliced into <2 mm thin cross-sections. Cross-sections from the

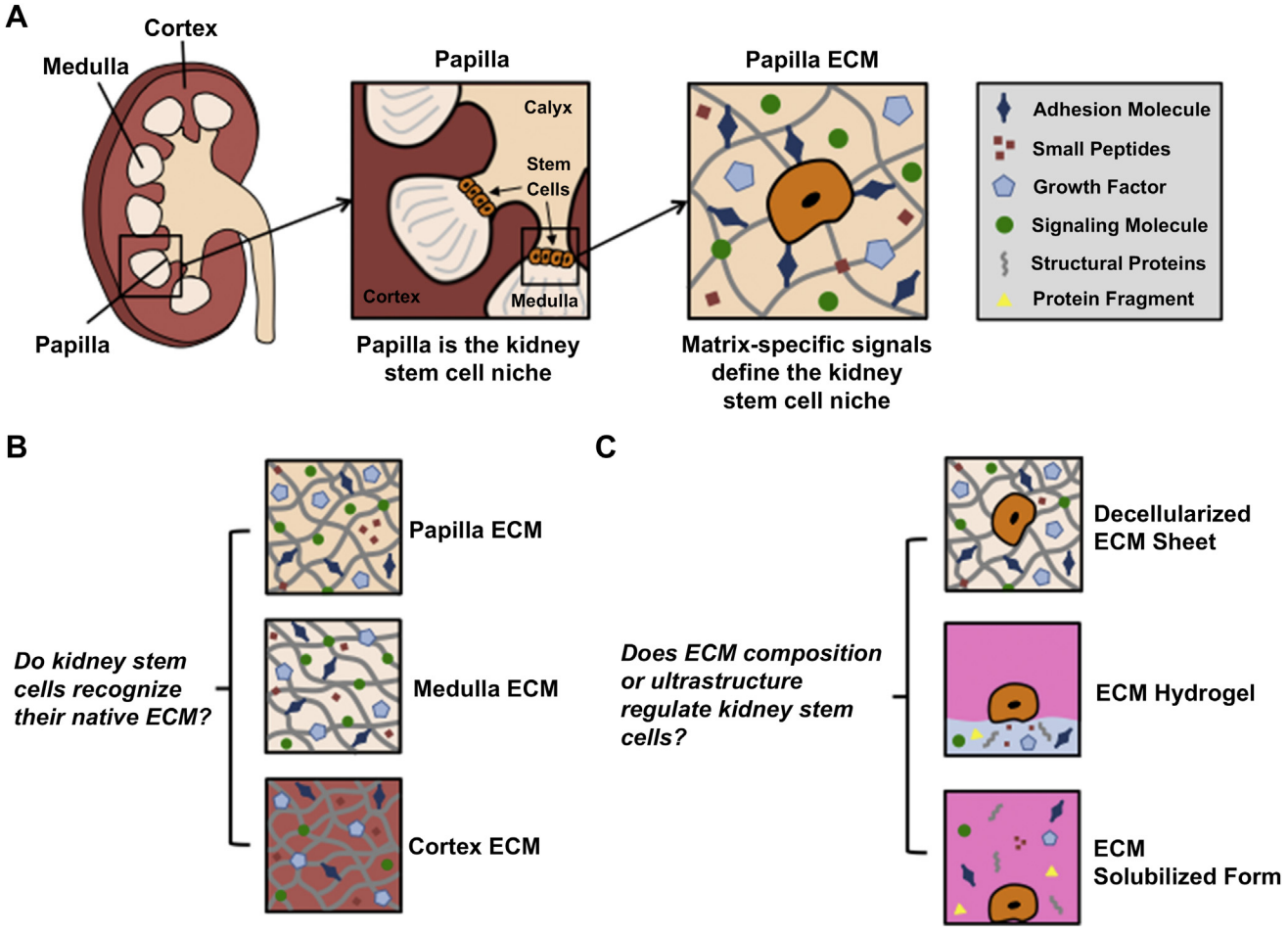

Fig. 1. Stem cell niche of the kidney. A The renal papilla is the stem cell niche in the kidney, defined by region-specific cues in the extracellular matrix of the papilla. B The renal cortex, medulla, and papilla were dissected and decellularized separately to obtain kidney region-specific ECM. C ECM was prepared in sheets, hydrogels, and solubilized forms for cultivation of two types of stem cells (kidney stem cells and mesenchymal stem cells). To obtain kidney region-specific ECM sheets, kidney regions were dissected before decellularization or the regional matrix was punched from decellularized whole kidney sections. Alternatively, pre-sectioned regions were decellularized, snap-frozen in liquid nitrogen, ground into a fine powder, lyophilized, and pepsin-digested to yield kidney region-specific ECM digests that were neutralized to obtain solubilized ECM forms. By treating these digests with salt, base, and heat, we obtained kidney region-specific ECM hydrogels. Kidney stem cells (KSCs) that are native to the papilla were cultured on ECM sheets, hydrogels, or solubilized forms derived from the three kidney regions (cortex, medulla, papilla) and compared to mesenchymal cells (MSCs) cultured under the same conditions.