Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

Biomaterials 28 (2007) 4806–4817

Biomaterials

www.elsevier.com/locate/biomaterials

The effect of hyaluronic acid size and concentration on branching morphogenesis and tubule differentiation in developing kidney culture systems: Potential applications to engineering of renal tissues

Eran Rosines[a], Heidi J. Schmidt[a], Sanjay K. Nigam[b,c,d,*]

[a]*Department of Bioengineering, University of California, San Diego, 9500 Gilman Drive #0693, La Jolla, CA 92093-0693, USA*
[b]*Department of Pediatrics, University of California, San Diego, 9500 Gilman Drive #0693, La Jolla, CA 92093-0693, USA*
[c]*Department of Cellular and Molecular Medicine, University of California, San Diego, 9500 Gilman Drive #0693, La Jolla, CA 92093-0693, USA*
[d]*Department of Medicine, University of California, San Diego, 9500 Gilman Drive #0693, La Jolla, CA 92093-0693, USA*

Received 26 April 2007; accepted 19 July 2007

Abstract

Hyaluronic acid (HA) is a glycosaminoglycan of tissue engineering importance that plays a vital role in mammalian development. *In vitro* kidney culture methods were utilized to investigate the importance of HA during renal organogenesis. We found that HA has the ability to simultaneously modulate ureteric bud (UB) branching, promote mesenchymal-to-epithelial transformation, and promote differentiation of both metanephric mesenchyme (MM) and the UB depending on the concentration and molecular weight (MW) of HA. Hyaluronidase inhibited branching morphogenesis in both isolated UB and whole kidney cultures, suggesting endogenous HA is required for branching morphogenesis. HA exhibited morphogen-like properties, stimulating branching morphogenesis at low concentrations (0.1%) and low MW (6.55 kDa), but inhibiting at high concentrations (3.75%) and high MW (234.4 kDa). Furthermore, HA of every MW tested promoted collecting duct differentiation as measured by AQP-2 expression. E-cadherin immunostaining and qPCR of nephron differentiation markers (OAT-1, NaP_i-2, AQP-1, and THP) demonstrated that HA of a variety of MWs strongly promotes mesenchymal epithelialization and nephron differentiation in a concentration-dependent manner. Since the HA synthesis and degradation genes, *has-2* and *hyal-2*, are highly expressed during kidney development, this data suggests that specific sizes and concentrations of HA may act to independently regulate UB branching and promote tubular maturation, representing a potential switch for ending branching morphogenesis, as well as initiating nephron differentiation. In addition, the ability of HA to promote *in vitro* embryonic kidney growth and maturation, together with the biocompatibility and crosslinking capability of HA, suggests a potential use of HA for both creating an instructive, 3D scaffold for *in vitro* kidney engineering from developmental tissues, as well as promoting tubule regeneration in injured or cryopreserved kidneys.
© 2007 Elsevier Ltd. All rights reserved.

Keywords: Hyaluronic acid/hyaluronan; Molecular weight; Organ culture; Scaffold; Kidney tissue engineering; Kidney development

1. Introduction

Metanephric kidney development begins with the outgrowth of the ureteric bud (UB) from the Wolffian duct at week 5 in human development and approximately day 12 of rat development. The UB invades the surrounding metanephric mesenchyme (MM) and begins the process of branching morphogenesis. MM cells coalesce around the tips of the branching UB, thereby providing factors that promote UB branching morphogenesis and maturation, while the UB releases factors that promote epithelialization and differentiation of MM cells [1]. While many branching morphogenesis and mesenchymal-to-epithelial transformation (MET) regulatory factors have been identified [2–7], the mechanism(s) for stopping branching morphogenesis and beginning collecting duct and nephron differentiation is poorly understood. Here we demonstrate that hyaluronic

*Corresponding author. University of California, San Diego, 9500 Gilman Drive #0693, La Jolla, CA 92093-0693, USA.
Tel.: +1 858 822 3482; fax: +1 858 822 3483.
E-mail address: snigam@ucsd.edu (S.K. Nigam).

0142-9612/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2007.07.034

acid (HA) is a molecule that depending on size and concentration can modulate branching morphogenesis and promote differentiation of both the collecting duct and nephrons. Since the majority of nephron formation occurs in the phases of renal development following branching morphogenesis, HA represents a possible switch molecule that stops branching morphogenesis and promotes nephron differentiation.

HA is a simple polysaccharide consisting of repeating disaccharide units of D-glucuronic acid (1-β-3) *N*-acetyl-D-glucosamine (1-β-4) [8] synthesized by three HA synthase genes, *has-1*, *has-2*, and *has-3* [9]. *Has-2* has been demonstrated to be the most important of these genes responsible for at least 97% of HA synthesized during murine embryonic development and murine embryos lacking the *has-2* gene exhibit growth retardation evident by embryonic day 9 and lethality by day 9.5 (before UB outgrowth) [9]. Moreover, exogenous HA has been shown to improve the development of *in vitro*-produced bovine embryos and porcine parthenogenic embryos [10,11]. Together, this data indicates a vital role for HA during mammalian organogenesis and development. In renal organogenesis, HA has been implicated in the branching morphogenesis of UB cell lines and has been found to be expressed along with its receptor, CD44, along the basal surface of the branching UB [12]. Furthermore, HA has been demonstrated to be ubiquitously present throughout the interstitial space of the developing kidney [13]. However, much remains to be elucidated about the role of HA in kidney development.

Has-2 synthesizes HA chains between $2 \times 10^5 - 2 \times 10^6$ Da, yet HA is known to exist *in vivo* at much smaller molecular weights (MWs) and have different *in vivo* roles based on its MW [14]. The MW of HA is controlled *in vivo* by the degradative hyaluronidases (hyal). Although six different hyaluronidases are potentially involved in HA degradation, *hyal-1* and *hyal-2* are believed to be the primary functional hyaluronidases of mammalian somatic cells [15]. The different hyaluronidases play different roles in regulating HA; for example, *hyal-1* is a secreted protein and breaks down HA to a tetrasaccharide [16], while *hyal-2* exists as a cell-anchored protein and degrades HA to 20 kDa fragments [16,17]. If smaller MWs of HA (less than ~20 kDa) are regulatory molecules for kidney development, then *hyal-2* would be expected to be expressed during kidney development.

HA has been researched extensively for a wide variety of tissue engineering purposes (reviewed in [18]). HA is a non-antigenic, immunocompatible extracellular macromolecule [19,20] which has already demonstrated prevalent medical use as an injectible gel for viscosupplementation of osteoarthritic joints [21,22] and as a dermatological treatment for wrinkles [23–25]. Because of its hydroxyl and carboxylic acid groups, HA is easily modified to achieve particular characteristics and easily crosslinked to form hydrogels [26–31]. The ability of HA to promote *in vitro* embryonic kidney growth and maturation demonstrated here, together with the proven biocompatibility and crosslinking of HA, provide evidence for the potential use of HA towards creating an instructive, 3D scaffold for *in vitro* kidney engineering from developmental tissues.

In this study, we used *in vitro* models of kidney development to examine the role of HA in kidney development and its potential role as a scaffold material for *in vitro* kidney tissue engineering. The temporal expression of *has-2* and *hyal-2* during *in vivo* kidney development was characterized by qPCR. Next, hyaluronidase and HA of various MWs and concentrations were added to both *in vitro* metanephric kidney and isolated UB culture systems. Morphometric analysis was used to quantify the effects on branching morphogenesis. UB/MM differentiation was measured by quantitative PCR of functional renal differentiation markers. We found that HA simultaneously modulates UB branching morphogenesis, induces MET, and promotes differentiation in both MM and UB depending on the MW and concentration of HA.

2. Methods

2.1. Materials and reagents

Primers were synthesized by Allele Biotechnology (San Diego, CA). Hyaluronidase from *Streptomyces hyalurolyticus* was obtained from Sigma (St. Louis, MO). HA of the following MWs were obtained from Lifecore Biomedical (Chaska, MN): 234.4, 132.3, 64.0, 17.0, and 6.55 kDa. BSN cell-conditioned media (BSN-CM) was harvested as described previously [3]. Recombinant growth factors, fibroblast growth factor-1 (FGF1), and glial cell-derived neurotrophic factor (GDNF) were obtained from R&D Systems (Minneapolis, MN). Mouse anti-E-cadherin antibodies were from BD Biosciences Pharmingen (San Diego, CA) and goat anti-mouse AlexaFluor 594 was from Molecular Probes (Eugene, OR). FITC-conjugated *Dolichos biflorus* (DB) lectin was from Vector Laboratories (Burlingame, CA). Unless otherwise noted, all other reagents are from Sigma (St. Louis, MO).

2.2. Organ culture

Embryos from timed pregnant Holtzman rats (Harlan, Indianapolis, IN) at day 13 (E13) of gestation (day 0 being the day of appearance of the vaginal plug) were dissected free of surrounding tissues. The metanephric kidneys were isolated and placed on Transwell filters (0.4 μm pore size) (Costar, Cambridge, MA) in 12-well plates. The isolated kidneys were cultured at the media–air interface with 600 μL DMEM/F12 (GIBCO-BRL, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS) (Hyclone, Logan, Utah) and 1% antibiotics (GIBCO-BRL, Grand Island, NY) below the filter at 37 °C and 5% CO_2/100% humidity. When samples were treated with hyaluronidase, the enzyme was added to the media for a final concentration of 50 U/mL. HA was dissolved in sterile 0.2 μm filtered and autoclaved water (Hyclone, Logan, Utah) and then diluted to a 1× DMEM solution, buffered with HEPES (pH 7.2). Then, 270 μL of the HA solution were placed on top of the tissue sample for HA treatment. All treatments were present throughout the indicated culture period.

2.3. Isolated UB culture

UBs were dissected from E13 kidneys, suspended in a growth factor-reduced Matrigel (BD Biosciences, San Jose, CA) solution (1:1 Matrigel:DMEM/F12), and cultured with BSN-CM supplemented with 10% FBS, 1% antbiotics, 250 ng/mL FGF1, and 125 ng/mL GDNF as described previously [2,3,5,32–34]. The low growth factor condition

Download English Version:

https://daneshyari.com/en/article/10650

Download Persian Version:

https://daneshyari.com/article/10650

Daneshyari.com