Biochemical and Biophysical Research Communications 450 (2014) 1377–1382

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Two-way regulation between cells and aligned collagen fibrils: Local 3D matrix formation and accelerated neural differentiation of human decidua parietalis placental stem cells

Wen Li [a], Bofan Zhu [a], Zuzana Strakova [b], Rong Wang [a,*]

[a] Department of Biological and Chemical Sciences, Illinois Institute of Technology, 3101S Dearborn ST., Chicago, IL 60616, United States
[b] Department of Obstetrics and Gynecology, University of Illinois at Chicago, 820 S Wood Street, M/C 808, Chicago, IL 60612, United States

ARTICLE INFO

Article history:
Received 17 June 2014
Available online 5 July 2014

Keywords:
Collagen
Fibril alignment
Cell polarization
Neural differentiation
3D matrix
Atomic force microscopy (AFM)

ABSTRACT

It has been well established that an aligned matrix provides structural and signaling cues to guide cell polarization and cell fate decision. However, the modulation role of cells in matrix remodeling and the feedforward effect on stem cell differentiation have not been studied extensively. In this study, we report on the concerted changes of human decidua parietalis placental stem cells (hdpPSCs) and the highly ordered collagen fibril matrix in response to cell–matrix interaction. With high-resolution imaging, we found the hdpPSCs interacted with the matrix by deforming the cell shape, harvesting the nearby collagen fibrils, and reorganizing the fibrils around the cell body to transform a 2D matrix to a localized 3D matrix. Such a unique 3D matrix prompted high expression of β-1 integrin around the cell body that mediates and facilitates the stem cell differentiation toward neural cells. The study offers insights into the coordinated, dynamic changes at the cell–matrix interface and elucidates cell modulation of its matrix to establish structural and biochemical cues for effective cell growth and differentiation.

1. Introduction

Cell–matrix interaction plays a critical role in various physiological and pathological processes. Collagen type I represents one of the most abundant structural proteins that form the extracellular matrix (ECM) of vertebrates. It provides the mechanical stability for tissues and serves as a functional environment for cells [1,2]. Fibrillar collagen type I is typically aligned in parallel arrays in connective tissues, either locally or extensively [3–5]. Thus, it is attractive to construct aligned collagen fibril arrays to mimic the native tissue environment for *in vitro* studies. It has been reported that collagen molecules assemble into arrays of ordered fibrils when guided by the crystalline orientation of mica substrates [6,7]. The aligned matrices have been used to study β-1 integrin mediated cell adhesion, polarization, and migration. Regardless of cell type, it was observed that cells expressing α2β1-integrin are capable of dislocating the highly ordered collagen fibrils and depositing the loose fibrils around cell [4,8–10]. While it has been suggested that the 3D-like collagen matrix might induce specific signaling for cell development [4], it has not been studied explicitly.

In this study, we report on the two-way regulation between human decidua parietalis placental stem cells (hdpPSCs) and highly ordered collagen fibril array by directly monitoring the cell–matrix interaction via high-resolution AFM imaging. Since hdpPSCs are robust and easily derived, they are preferable for *in vitro* studies and clinical therapies [11,12]. In our previous study, we found that these cells are capable of neural differentiation on a collagen-coated substrate in a non-selective medium [13]. In this study, we probed the coordinated, dynamic cell–matrix interaction to reveal the matrix prompted cell polarization and cell prompted local 3D matrix formation. The concerted changes were found to accelerate neural differentiation of hdpPSCs.

2. Materials and methods

2.1. Collagen matrix preparation

Collagen type I solution (9.82 mg/ml) derived from rat-tail tendon was purchased from BD Biosciences. The solution in 0.1% acetic acid was diluted to 35 μg/ml in 10× PBS buffer containing 1 N NaOH to adjust the pH to 9 for effective collagen fibril assembly [13,14]. 400 mM KCl was added to promote collagen alignment on mica [7]. A drop of 30 μl collagen solution was cast on a freshly cleaved surface of a Muscovite mica disk (Grade V1, Ted Pella, Inc.,

* Corresponding author.
E-mail address: wangr@iit.edu (R. Wang).

http://dx.doi.org/10.1016/j.bbrc.2014.06.136
0006-291X/

Redding CA), and incubated at 37 °C overnight to achieve collagen gelation. After rinsed with PBS, the samples were subjected to AFM imaging at high resolution or serving as a matrix for cell culture. Blank plastic substrate, cut from a cell culture dish, was used as a control. Electro-spun (E-spun) collagen fibers were also prepared (see Supplementary Data), and were used in comparative studies to examine the cell response to 2D vs. 3D matrices.

2.2. Cell culture

Undifferentiated hdpPSCs (passage 2–3) were obtained from Dr. Strakova's lab and propagated in a self-renewal media according to their pre-defined protocol [12]. For differentiation experiments, the undifferentiated cells at passage 3–6 were plated at a density of 6000 cells/cm^2 on various matrices in non-selective, spontaneous differentiation medium (DMEM + 10% FBS + 1% non-essential amino acids).

2.3. Atomic force microscopic (AFM) imaging

AFM imaging was performed using a multimode Nanoscope IIIa AFM (Veeco Metrology, Santa Barbara CA), equipped with a J-scanner. Amplitude images of the aligned collagen matrices and the hdpPSCs were recorded in 1× PBS buffer in fluid tapping mode using Si_3N_4 tips at a resonance frequency of 8–10 kHz. hdpPSCs were gently fixed with 4% paraformaldehyde or ice-cold methanol for 3 min.

2.4. Immunofluorescence staining

A Nikon U-2000 microscope was used to collect the immunofluorescent images. The expression of F-actin, Collagen-I and β1-integrin were tracked at 6–32 h post-plating. The expression of β3-tublin and Neu-N were examined in cells at Day 1 and Day 5 of differentiation. The primary antibodies used in this study include mouse anti-F-actin (Millipore, Temecula CA, 1:100 dilution), rabbit anti-collagen-I (Abcam, Cambridge, MA 1:100 dilution), rabbit anti-βIII-Tublin (Tuj1, Abcam, 1:200 dilution), mouse anti-NeuN (Millipore, 1:100 dilution) and rabbit anti-β1-integrin (Santa Cruz Biotechnology, 1:100 dilution). Secondary antibodies were purchased from Invitrogen (Carlsbad, CA) and used at a dilution of 1:200.

The images were quantitatively analyzed by ImageJ (NIH). In determining the percentage of positively stained cells, we set the threshold at 50% of the highest staining intensity across the samples. Cell length-to-width ratio was analyzed to reveal cell polarization. Since cell width varies along an elongated cell, average cell width was evaluated from the ratio of cell area and cell length.

3. Results

3.1. Collagen fibril alignment and matrix induced cell polarization/differentiation

As shown in the AFM image in Fig. 1A, collagen molecules are assembled into collagen fibrils of 6 ± 1 nm in diameter and 3.4 ± 0.4 μm in length. They exhibit unidirectional alignment and uniform coverage across the mica substrate. The characteristic collagen D-period of 68 ± 1 nm is highlighted in the inset of Fig. 1A, and is consistent with our previous observations [13,14]. A blank plastic substrate is rather smooth with a maximum height difference of 4 nm, despite the observed line scratches (Fig. 1B). We monitored hdpPSC elongation in 2–32 h post-plating on collagen/mica and plastic, and the changes in length-to-width ratio, derived from optical images (Fig. 1C, D and Fig. S1), were summarized in Fig. 1G. Within 2 h post-plating of hdpPSCs on a collage/mica matrix, many cells have started to elongate. All cells are bipolar after 18 h post-plating (Fig. 1C). However, cells on plastic appear non-polar in shape (Fig. 1D). With extended cell culture to Day 5, while cells on plastic show random shapes (Fig. 1F), most cells on collagen-mica appear with long filaments extending from a small cell body (Fig. 1E), resembling neural progenitors [15,16]. Thus, we examined the neural differentiation profile of hdpPSCs. The staining in Fig. 1E and F was against βIII-Tublin (red), a neuron-specific isoform expressed by immature neurons and outgrowth neurites, and NeuN (green), expressed by neural progenitors and mature neurons [17,18]. Results from quantitative analysis of the staining data (Fig. 1H and I) indicate that βIII-Tublin positive cells are predominant on collagen/mica even on Day 1 of differentiation. These cells are mostly NeuN negative and express βIII-Tublin at a high level. By Day 5 of differentiation, NeuN positive cells become dominant (69%). Importantly, these cells express NeuN at a substantially higher level and express βIII-Tublin at a relatively lower level, suggesting successful differentiation and maturation of the polarized hdpPSCs toward neurons [18]. On the contrary, nominal cells on plastic are NeuN positive by Day 5 of differentiation even though the level of βIII-Tublin expression has increased. For comparison, we also prepared aligned collagen fibers by electrospinning method. These fibers are much thicker (~1 μm in diameter), and similarly promote cell polarization as collagen fibrils do on mica. However by Day 5 of differentiation, the cells on E-spun fibers express NeuN at a significantly lower level despite a higher level expression of βIII-Tublin. The results from the current study corroborate a dramatically faster neuronal differentiation and maturation process on collagen/mica substrates.

3.2. Examination of cell–collagen fibril interaction at high-resolution

AFM images in Figs. 2 and 3 provide insights into the cell–collagen fibril interaction through the extended lamellipodia and filopodia. Cells use sheet-like lamellipodia and finger-like filopodia to probe the environment for cues, function as pathfinders, and play critical roles in cell adhesion and migration [19–21]. On collagen/mica substrates and before a cell is fully differentiated, long high-density filopodia extend from both ends of a polarized cell preferentially along the direction of collagen fibril alignment (Fig. 2A). In contrast, cells on a plastic substrate show a number of filopodia at the periphery of a cell in random directions (Fig. 2B and Fig. S2). It is known that filopodia and lamellipodia bind to collagen through collagen–β1-integrin specific interaction to form focal adhesion complexes, which assist in targeting the location of actin filaments and signaling components [19,21]. The aligned collagen fibrils are expected to coax the deposition of focal adhesion complexes that cause the cytoskeleton structure to stretch [3,5,22], leading to cell polarization and migration along the collagen fibrils (see Fig. 4).

As shown in Fig. 2C, when a cell elongates along the collagen fibrils, fibril alignment near the cell is disrupted. The originally orderly assembled and densely packed fibrils are pulled toward the cell (Fig. 2D), causing the fibrils to split and separate from the well-organized fibril network (white circles in Fig. 2F). This is consistent with others' reports that cells are able to dislocate and peel collagen fibrils from mica substrates [4,8,9], suggesting a weaker interaction between collagen and mica than between collagen and a cell. Presumably, when a cell deforms during polarization, the cytoskeleton exerts a pulling force to the collagen matrix in the direction perpendicular to cell polarization while the cell membrane withholds the collagen fibrils through the collagen–integrin interaction. The pulling force is sufficiently high to drag the collagen fibrils toward the cell, disrupt the fibril network,