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Research Article

Multiple immunophenotypes of cardiac telocytes

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ABSTRACT

Aims: Telocytes (TCs) form a 3-dimensional network in the myocardial interstitium, which most probably play important role(s) in heart development. However, the dynamics of their prolongations, continuous cell shape changes and adherence properties have not been well documented till recently. The aim of this study was to investigate dynamics of extension of prolongations (Telopods) and multiple phenotypes of cardiac TCs cultured *in vitro*.

Methods: Cardiac TCs were isolated from neonatal rats by a combined enzyme digestion process and identified by light microscopy, immunofluorescence analysis and scanning using electron microscopy (SEM). Their continuous changes in shape were analyzed by a Live Cell Imaging System and multiple phenotypes were identified by immunofluorescence analysis using various markers, like vimentin, c-kit, CD34, nanog and sca-1.

Results: Cardiac TCs displayed piriform/spindle/triangular shapes with long and slender telopodes showing extremely long prolongations. The morphology of cell body was continuously changing while their prolongations were extending gradually. After adhering to the surface, TCs' movement and extension of their prolongations lasted for approximately 1.5 h. Cardiac TCs expressed mesenchymal cell marker vimentin, hematopoietic stem cell marker CD34, embryonic stem cell-associated gene of Nanog, and myocardial stem cell markers sca-1 and c-kit.

Conclusion: These findings indicate that cultured TCs *in vitro* have multiple phenotypes, which are most likely important for evaluating their functional roles in heart development.

1. Introduction

Telocytes (TCs) are described as interstitial cells with extremely long and thin prolongations, called telopodes (Tps) (www.telocytes.com). Tps have dilated portions (podoms) and very thin segments (podomeres), exhibiting moniliform appearance [1,2]. These unique structural characteristics evoked substantial interest in terms of their functional significance [3–9]. However, dynamics of Tps extension, cell shape changes and adherence have not been well understood.

Cardiac TCs have been identified in epicardium [10], myocardium [11], endocardium [12], subendocardium [13], stem cell niches [14], myocardial sleeves [15] and heart valves [16]. They form an interstitial network that plays a very important role in heart development, renewal and repair [17,18]. Intramyocardial transplantation of TCs has potential to reduce myocardial infarction and improve cardiac function in rats [19].

To date, specific molecular markers were not available to detect TCs, so morphology identification using transmission electron microscopy (TEM) remains as only firm diagnostic tool for detection of TCs [2,20]. In this study, cardiac TCs were identified by light microscopy and scanning electron microscopy (SEM). The phenotype of TCs was characterized by CD34, c-kit, vimentin and caveolin-1 positivity [21]. TCs in different tissues display different phenotypes [22,23]. Cardiac TCs express vimentin, CD34 and PDGFR-β [16]. The present study was to investigate dynamics of telopdes and features of their multiple immunophenotypes. The findings in this study may provide the direct evidence of the morphology of the cardiac TCs and a theoretical background to explain the role of TCs in heart development and myocardial repair following cardiac injury.

2. Material and methods

2.1. Animals

Neonatal Sprague-Dawley rats (1–3 day-old, 7.2 ± 0.35 g, $n=20$) were utilized in this study. Animal treatment was performed according to the guidelines of The Ministry of Science and

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Technology of the People's Republic of China [(2006)398] and approved by the Xinxiang Medical University Animal Care Committee (No. 030032).

2.2. Isolation and culture of cardiac TCs

Cardiac TCs were isolated from neonatal rat hearts. Briefly, heart tissues were minced into small pieces of about 1 mm^3 after washing with sterile phosphate-buffered saline (PBS). Minced tissues were incubated with 0.1% collagenase I/0.125% trypsin (Sigma-Aldrich, St. Louis, MO, USA) at 37 °C for 10 min. The suspension was collected, and the collagenase and trypsin were inactivated by adding an equal volume of Dulbecco minimum essential medium–low glucose (DMEM-LG) to the suspension. This process was repeated 3–5 times until the tissue blocks were completely digested. Next, the suspension was centrifuged at 1200 r/min for 5 min. The cellular pellet was filtered through a 40 μm cell strainer (BD Biosciences, San Jose, CA) and was resuspended in DMEM-LG culture medium supplemented with 10% fetal calf serum, 100 U/ml penicillin and 100 mg/ml streptomycin (Sigma-Aldrich). The cells were plated in dishes and cultured in a CO_2 incubator at 37 °C. After 60 min, suspensions were plated in 6-well plates to remove fibroblasts. When cells reached 85% confluence, the cells were harvested for further passage following 0.25% trypsin/0.01% EDTA digestion (Amresco, Solon, Ohio, USA). The morphology of TCs were viewed and imaged with a phase contrast microscope.

2.3. Morphology analysis

Cell morphology was analyzed on sub-confluent cell monolayer at 100 × magnification under a phase-contrast microscope (Leica). The pictures were taken with a Kodak camera. Cell viability was assessed using Trypan blue staining.

2.4. Ultrastructure analysis

Cardiac TCs grown on coverslips were fixed with 3% glutaraldehyde in PBS (pH 7.2) for 30 min at room temperature, and then washed with PBS three times. The samples were then dehydrated through graded ethanol (30%, 50%, 70%, 85% and 90%) and dried for 6 h at room temperature. The dried samples were pre-processed with vacuum deposition of gold plating, and then imaged by a FEI Quanta200 environmental scanning electron microscope (FEI Co., Hillsboro, OR, USA).

2.5. Dynamic morphology analysis

Cardiac TCs were cultured in 6-well plates. The dynamic morphology was analyzed with a Live Cell Imaging System (xcellence, Olympus, Japan). Parameters were set as follows: 1 image/min, duration time 12 h.

2.6. Immunofluorescence staining

Cardiac TCs grown on coverslips were fixed with 4% paraformaldehyde (Sigma-Aldrich) for 20 min, subsequently washed with PBS three times, and treated with 0.3% Triton X-100 in PBS for 15 min at room temperature. The samples were then blocked with 2% BSA for 30 min, and incubated with the primary antibodies (Mouse anti-c-kit, Santa Cruz, 1:200; Rabbit anti-vimentin, Abcam, 1:100; Rabbit anti-CD34, Bioss, 1:300; Mouse anti-vimentin, ZSGB-BIO, 1:50; Rabbit anti-Nanog, Santa Cruz, 1:100; Rabbit anti-Sca-1, Millipore, 1:200; Mouse anti-cTnT, Abcam, 1:500) at 37 °C for 2 h. The samples were subsequently incubated with FITC- or Cy3-conjugated secondary antibodies and 4, 6-diamidino-2-phenylindol dihydrochloride (DAPI), and viewed under a Nikon Eclipse 80i microscope (Nikon Instech Co.). All experiments were performed in triplicates from different culture preparations. Control staining was performed without primary antibody.

3. Results

3.1. Morphology of cardiac TCs

The isolated cells from rat hearts in suspension showed small and round shapes. Cardiac fibroblasts attached to the flasks after plating for 30–60 min, TCs attached after plating for 60–90 min, and cardiomyocytes attached after plating for 90 min by differential velocity adherence technique. Most of the attached cells were mainly cardiac fibroblasts and cardiomyocytes that presented spindle, oval and triangle shapes (Fig. 1). A few cells were TCs that exhibited small prominent body with 1–7 very thin and extremely long (10–1000 μm) prolongations, also named telopodes (Tps) that consisted of the alternating dilated podomeres and thin podoms. TCs contacted cardiomyocytes, fibroblasts and other cell types though their TPs (Fig. 1). Because of these unique structural characteristics, TCs were relatively easy to distinguish from cultured fibroblasts and other interstitial cells that maintained spindle-like and stellate shapes *in vitro* culture (Fig. 1). After plating for 48 h, TCs proliferated rapidly and reached the logarithmic growth phase (Fig. 1C). They reached confluence after plating for 6–7 days.

3.2. Live Cell Imaging

Dynamic morphology and movement of TCs were recorded by a

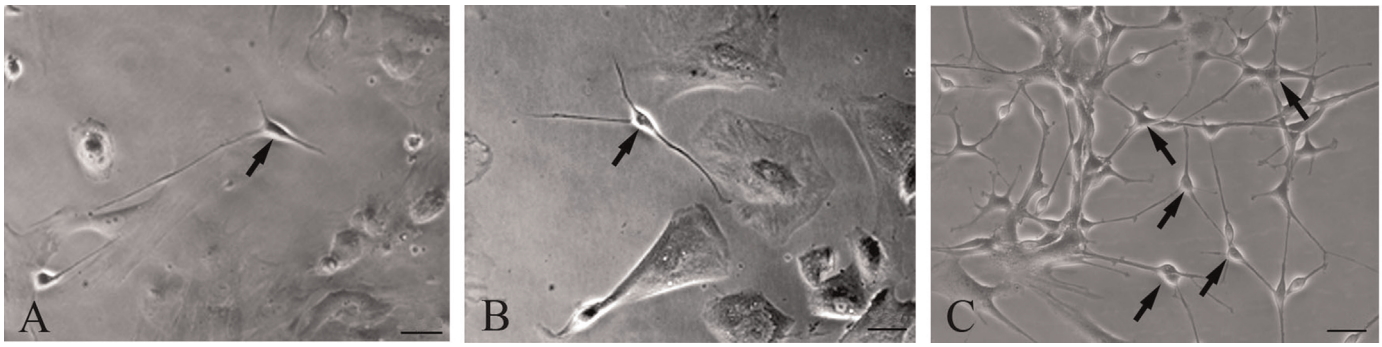

Fig. 1. Morphology of cardiac TCs under phase-contrast microscope. (A) Piriform or triangular and small cell bodies; TC with single telopodes (Tp) and the other with three slender Tps that are in contact with cardiomyocytes. (B) TC shows spindle and small cell body with three slender telopodes that interact with two cardiac fibroblasts. (C) Most TCs have prominent bodies and their Tps have two branches, forming a network in an exponential or log phase of growth. Scale bar = 25 μm.