Comparison of Young and Old Cardiac Telocytes Using Atomic Force Microscopy

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Abstract

Aims: Investigation of surface morphology of young and old cardiac telocytes using atomic force microscopy (AFM). Methods: Young female SD rats (3-month-old) and old female SD rats (24-month-old) were used for the isolation of cardiac telocytes (CTs). The isolated CTs were analyzed by immunofluorescent staining for CD34 and c-Kit, and then were applied for AFM analysis. Results: The unique morphology and c-Kit/CD34 positive well confirmed that the isolated cells belong to CTs. The results of AFM revealed that the mean of cell body length and mean of telopode length of young CTs were longer than those of old CTs. The mean of cell surface roughness and mean of telopode surface roughness of young CTs were smaller than that of old CTs.

Keywords

Cardiac telocytes; Aging; Atomic force microscopy; Surface roughness.

1. Introduction

A novel cell population of cardiac interstitial cells, named telocytes, has recently been identified in the heart interstitium. Cardiac telocytes are observed in myocardium with unique morphology, a piriform, spindle or triangular shape with a nucleus that occupies approximately 25-30% of the cell volume and very long, thin, dichotomously branched protrusions with a moniliform aspect called telopods, which are considered the 'ultrastructural hallmark' that distinguishes CTs from other interstitial cells (such as cardiac fibroblasts). At present, there is no specific marker for telocytes, the expression of c-Kit and CD34 as well as PDGFRα are applied to identify CTs [1].

The distribution of CTs in the heart is among the atrium, ventricle, endocardium, heart valve and epicardium. CTs form a crisscross network structure in the myocardial interstitium and play a supporting role to other cardiac cells [2]. It was reported that transplantation of CTs in infarcted myocardium is able to improve regeneration of heart after myocardial infarction (MI) [3]. All these suggest that CTs play an important role in physiopathology of myocardium. By far, the age-related changes of CTs in cell surface and the morphology are not yet investigated. It is believed that understanding of age-related changes of CTs in cell surface and their morphology will benefit us to uncover the mechanism of CT aging. AFM works by measuring the atomic interaction between the surface and AFM scanning probe with a sensor is able to scan image of measured surface. Therefore,

AFM technology is powerful technique to analyze surface structure and characteristics. Present study is therefore designed to address these interesting issues using AFM.

2. Methods

2.1 Main reagents and instruments

Fetal bovine serum, 2.5g/L trypsin, high glucose DMEM medium (Gibco, USA); c-Kit rabbit polyclonal antibody (Novus: NBP1-19865); CD34 goat polyclonal antibody (R&D: AF4117); Anti-Rabbit IgG microbeads (miltenyibiotec); DM4000B fluorescence microscope (LEICA); IX51 fluorescence phase inversion microscope (Olympus); CP Research atomic force microscope (Thermo microscopes);Cy3-conjugated donkey anti-goat Proteintech (SA00009-2); FITC-conjugated donkey anti-rabbit IgG Proteintech (SA00003-8).

2.2 Experimental Animals

Young SD female rats (3-month-old) and old SD female rats (24-month-old) were housed for 2 weeks to allow them to adapt before experimentation. Food and water *ad* libitum were feed to rats. Animal care, surgery and handling procedures were performed according to regulations set by The Ministry of Science and Technology of the People's Republic of China ([2006] 398) and approved by Jinan University Animal Care Committee.

2.3 Isolation and cultivation of young and old Telocytes

Young and old SD female rats were used for the isolation of CTs as previous reports [3,4]. Briefly, the hearts were isolated and minced, and then the tissues were treated with 2.5 ml of DMEM+ 0.05% collagenase P and 0.1% trypsin at 37°C on a shaker (180 rpm) for 10min. After the suspension was removed, collagenase and trypsin medium were added and the mixture was incubated at 37°C on a shaker for 45 min. The digested tissue was dissociated by pipetting gently every 15 min. The pellet was re-suspended in PEB medium. The collected supernatant was further centrifuged. The cell pellet was then mixed with PEB and rabbit anti-rat C-Kit antibody at 4°C for 60 min. An additional PEB was then added, the mixture was centrifuged. The pellet was re-suspended in PEB, and goat anti-rabbit IgG-microbeads was added, incubation at 4°C for 25 min. The mixture was next added to an MS columnin a magnetic field, and the unlabeled cells were allowed to pass through. The isolated cell pellet was collected after centrifugation by culture in DMEM containing 20% fetal calf serum at 37°, 5% CO2 and 95% air incubator. It was established that using the method more than 93% of the isolated cells were C-Kit$^+$ and CD34$^+$. Only passage 5 or less isolated CTs were used for experimentation.

2.4 CD34 and c-Kit immunofluorescent staining

The cultured CTs were fixed with 4%(w/w) paraformaldehyde in phosphate buffer saline for 10min.After incubation with 5% normal goat serum for 1 h to block non-specific staining, cell were incubated with polyclonal rabbit anti-c-Kit antibody overnight, followed by FITC-conjugated donkey anti-rabbit IgG incubation for 1 h at room temperature. After c-Kit staining, the cells were incubated with goat anti-CD34 overnight and then with Cy3 conjugated donkey anti-goat IgG. All of the antibodies were diluted in 0.1% Triton X-100 in PBS (pH 7.4). Control cells were prepared by omitting primary antibodies. Pictures were captured using a microscope with excitation wavelengths appropriate for Cy3 and FITC [5].

2.5 Atomic force microscopy analysis

The isolated CTs were cultured at 37℃(5%CO_2) in containing 20% fetal bovine serum. The cells were cultured for 24h before experiments. When the cells density reached 50%, the culture medium was removed, washed three times with 1xPBS. After fixation with 4% (w/w) paraformaldehyde, the stationary solution was discarded, washed three times with ultra-pure water, dried and preserved at room temperature, and then analyzed by atomic force microscopy. The average length of the cell

body is calculated by the distance of the farthest point at both ends of the cell body parallel to the direction of the cell body. While, the average length of the telopode is calculated by the distance between the farthest points parallel to the telopode and at both ends of the telopode. Cell body roughness is measured by a scan (10×10μm) on cell surface of cell body. For roughness analysis of telopode, five scans (each scan: 3×3μm) were conducted on telopode randomly to measure the roughness. The mean roughness of young and old telopode was applied to comparison.

3. Result

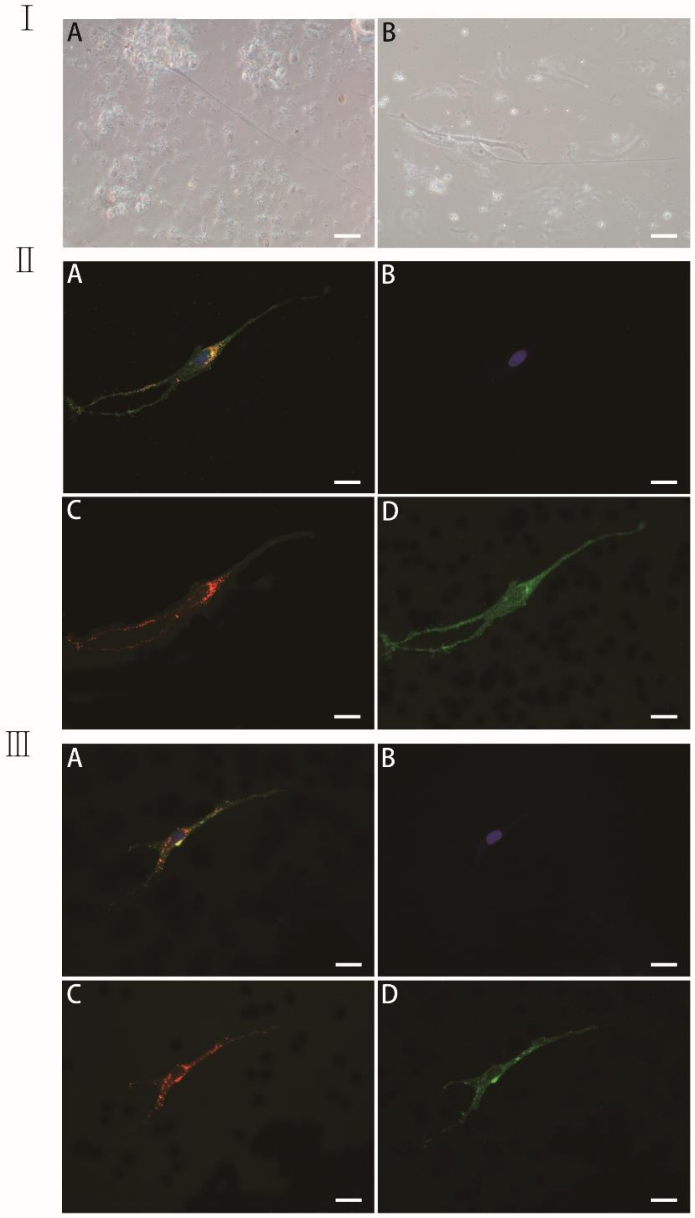

Figure1: Ⅰ： Phase contrast microscopy images of isolated CT with its unique morphology, spindle shape with thin and very long prolongations included telopode podomers and podoms, ranging from tens to hundred μm. (A) Cultured young isolated CTs. (B) Cultured old isolated CTs. Ⅱ: Double immunofluorescent staining for CD34 and c-Kit in young CTs cultured for 3 days. (A) merge of B (DAPI), C (c-Kit) and D (CD34). Ⅲ： Double immunofluorescent staining for CD34 and C-Kit in old isolated CTs cultured for 3 days. (A) merge of B (DAPI), C (c-Kit) and D (CD34). Scale bar = 50 μm.

3.1 Confirmation of surface markers and unique morphology of isolated cardiac telocytes

The isolated young and old cells were long spindle shape with thin and very long prolongations, ranging from tens to hundred μm. The immunofluorescent staining for c-Kit and CD34 documented that young and old isolated cells are c-Kit/CD34 positive. The unique morphology and c-Kit/CD34 positive well confirmed that the isolated cells belong to cardiac telocytes (Figure 1).

3.2 Atomic force microscopy analysis of young and old cardiac telocytes

The atomic force microscopy was applied to analyze and measure the young and old CTs (Figure 3A and 3C). It was found the cultured young CTs were usually spindle shape with longer telopode comparing with old CTs. In addition, the surface of cell body and telopode of young CTs were smoother than those of old CTs (Figure3B and 3D). The measurement further showed that the mean of cell body length and mean of telopode length of young CTs were 53±22μm and 73±25μm. The mean of cell body surface roughness and the mean of telopode surface roughness of young CTs were 181±131nm and 46±10nm. While, in old CTs, the mean of cell body length, mean of telopode length, mean of cell body surface roughness and mean of telopode surface roughness were 55±11μm, 58±38μm, 325±251μm and 86±21μm. The comparison of above parameters between young and old CTs was listed in Table 1. It was found that the mean of cell body length and mean of telopode length of young CTs were longer than those of old CTs. The mean of cell surface roughness and mean of telopode surface roughness of young CTs were smaller than that of old CTs (Figure 2 and3).

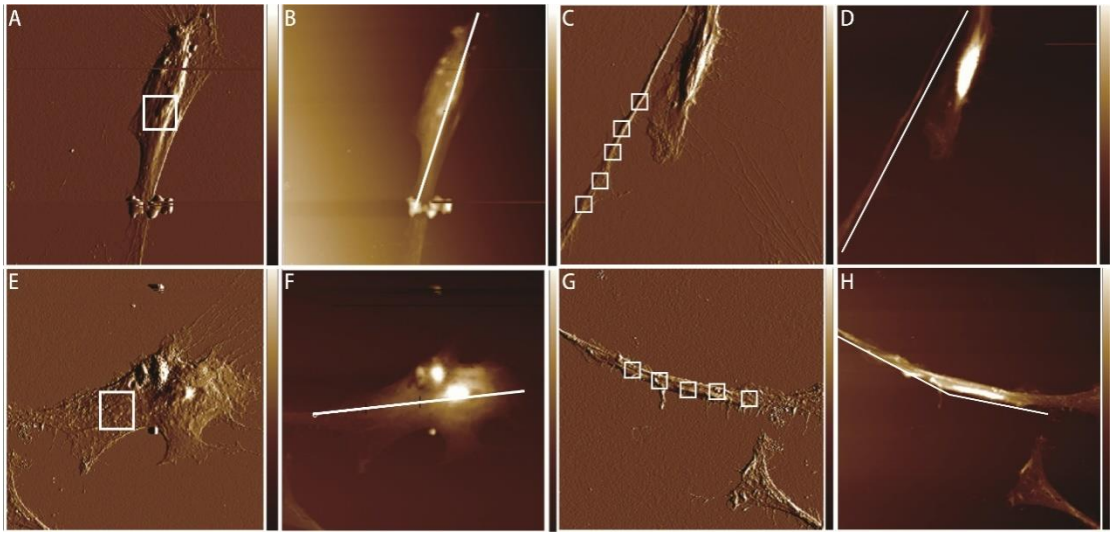

Figure 2: Measurement of AFM scan in young and old CTs. (A) AFM image of cell body of young CTs. The white square (10×10μm) indicates location of scan for roughness analysis. (B) Measurement of cell body length of young CTs. (C) AFM image of telopode of young CTs. The white square (3×3μm) indicates location of scan for roughness analysis. (D) Measurement of telopode length of young CTs. (E) AFM image of cell body of old CTs. The white square (10×10μm) indicates location of scan for roughness analysis. (F) Measurement of cell body length of old CTs. (G) AFM image of telopode of old CTs. The white square (3×3μm) indicates location of scan for roughness analysis. (H) Measurement of telopode length of old CTs.

Table 1 The comparison of young CTs and old CTs using AFM

	Cell body length (μm)	Telopode l ength (μm)	Cell body surface roughness (nm)	Telopode surface roughness (nm)
Young CTs (N=4)	55±22	73±25	181±131	46±10
Old CTs (N=10)	53±11	58±38	325±251	86±21

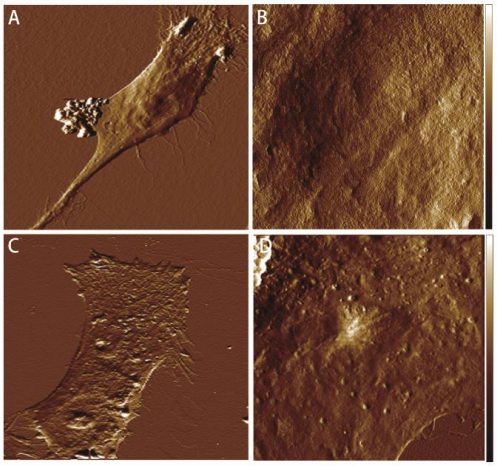

Figure 3: The AFM morphology of young CTs and old CTs. (A) The representative AFM scan image of young cultured CTs. (B) AFM microscopy quantification of surface roughness of (A). (C)The representative AFM scan image of old cultured CTs. (D) AFM microscopy quantification of surface roughness of (C).

4. Conclusion

It was found that the mean of cell body length and mean of telopode length of young CTs were longer than those of old CTs. The mean of cell surface roughness and mean of telopode surface roughness of young CTs were smaller than those of old CTs. It suggests that aging of CTs will incur the morphology changes. During aging, the lengths of cell body and telopode of CTs trend to become shorter, while, the roughness of cell body and telopode become more intense. These aging changes might relate to functional changes of aged cardiac telocytes. The underlying mechanism needs to be studied deeply in future.

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