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Research Article

Identification of Stem-Like Cells in Atrial Myxoma by Markers CD44, CD19, and CD45

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Atrial myxoma is the most frequent tumor arising mainly in atrial septum and its origin remains uncertain. It has been reported that a subpopulation of stem-like cells are present in benign tumors and responsible for tumor initiation and maintenance. In this study, we investigated whether stem-like cells could contribute to the atrial cardiac myxoma. Immunohistology data confirmed that a population of cells bearing the surface markers CD19, CD45, and CD44 resided in a mucopolysaccharide-rich matrix of myxoma. Moreover, we isolated myxoma cells with phase-bright culture method and confirmed that myxoma derived cells express robust level of CD19, CD45, and CD44. Furthermore, the pluripotency of this population of cells also was validated by cardiomyocytes and smooth muscle cells differentiation in vitro. Our results indicate that primary cardiac myxoma may arise from mesenchymal stem cells with the ability to generate tumors with multilineage differentiation. In conclusion, this study for the first time verified that stem-like cells are present in atrial myxoma and this population of cells may have the capacity for myxoma initiation and progression.

1. Introduction

Cardiac myxoma is the most frequent tumor of the heart [1]. It is characterized by myxoid appearance of a mucopolysaccharide-rich extracellular matrix and appears to be qualitatively distinct from different cases of histopathology. Previous studies indicated that myxoma cells arise from remnants of subendocardial vasoformative reserve cells or multipotential primitive mesenchymal cells in the fossa ovalis and surrounding endocardium, which can differentiate into a variety of cell lineages including endothelial, fibroblastic, hematopoietic, glandular, neurogenic, and smooth muscle cells [1–3]. Histopathology analysis data revealed that myxoma is mainly composed of stellate, fusiform, or elongated cell incorporated into myxoid matrix [2] and phenotypic characterization studies indicate that the origin of myxoma is from pluripotential cells [1].

The histogenesis of cardiac myxoma remains unclear and different cell phenotypes, including epithelial, endothelial, myogenic, myofibroblastic, and neural cells, were observed in myxoma by immunohistology [4]. Recent studies have indicated that cancer stem cells, a type of cancer cell that can self-renew and differentiate into multiple cell types, are responsible for tumor initiation, recurrence, and metastasis [5, 6]. Though the malignant potential of cardiac myxoma remains doubtful, a possible explanation for histogenesis of myxoma is that a population of cells with pluripotency is the origin.

It is believed that the myxoma cells are from primitive multipotent mesenchymal cells [7]. To date, a number of putative markers for cancer stem cell have been reported and CD44 is the most common cancer stem cell surface marker [5]. Adult cardiac stem cells in the myocardium have been identified using a variety of approaches, including physiological properties such as the ability to form multicellular spheroids [8]. The pattern of protein and gene expression indicates that myxoma cells may be phenotypically similar to a more primitive cardiac progenitor or primordial cardiac stem cells [1]. In this study, we isolated myxoma cells by sphere-forming methods. Moreover, the expression of cancer stem cell marker CD44 and the differentiation of myxoma derived cells were investigated.

2. Materials and Methods

2.1. Tissue Collection. Atrial myxomas were obtained from surgical specimens from the Department of Cardiothoracic Surgery, TEDA International Cardiovascular Hospital, Tianjin, China. Three patients, all female, were 52, 55, and 56 years old, respectively. And all participants gave written informed consent.

2.2. Histological Analysis. To investigate the histology of myxoma, samples were fixed in 4% paraformaldehyde, cut transversely, embedded in paraffin, and stained with hematoxylin and eosin (H&E). To carry out immunohistology, atrial myxomas were embedded into OCT compound (Miles Scientific) and cut into transverse sections at 5 μm thickness. To explore if myxomas are CD19, CD45, and CD44 positive, anti-CD19, anti-CD45, and anti-CD44 antibodies (all from BD Pharmingen, Mountain View, CA) were used. Alexa Fluor 488 and Alexa Fluor 594-conjugated secondary antibodies were applied appropriately (Invitrogen, Carlsbad, CA). DAPI was used for nuclear counterstaining.

2.3. Cell Cultures. To investigate if myxoma cells can be cultured, tissues were cut into 1 to 2 mm piece, washed with Hanks' balanced salt solution (HBSS) (Invitrogen, Carlsbad, CA), and incubated with 0.1% collagenase II for 30 minutes at 37°C with frequent shaking [8]. Cells were then filtered through 100 μm mesh. The obtained cells were cultured in Iscove's Modified Dulbecco's Medium (IMDM) supplemented with 10% fetal bovine serum (FBS) (Hyclone, Logan, UT), 0.1 mM nonessential amino acids, 100 U/mL Penicillin G, 100 μg/mL streptomycin, 2 mmol/L glutamine, and 0.1 mmol/L β-mercaptoethanol [8]. After 2 to 3 weeks, a population of phase-bright cells appeared over the adhered fibroblast-like cells. These phase-bright cells were collected by two washes with PBS and one wash with cell dissolution buffer (Gibco, Grand Island, NY) at room temperature under microscope monitoring and subcultured with the same medium [8]. For cardiac and smooth muscle differentiation, myxoma derived cells were cultured under conditions as previously described [8, 9].

2.4. Flow Cytometry Analysis. Flow cytometry analysis of the myxoma derived cells and the subcultured cells was carried out. Antibodies used in this study were phycoerythrin (PE) conjugated anti-CD44 and allophycocyanin (APC) conjugated anti-CD45 and anti-CD19 (all from BD Pharmingen). The stained cells were analyzed using FACS LSR (Becton-Dickinson, MA). Dead cells stained by propidium-iodide (PI) were excluded from the analysis. Isotype-identical antibodies served as controls (BD Pharmingen). FlowJo software (Tree Star Inc., Ashland, OR) was used for followed data analysis.

3. Results and Discussion

3.1. Pathology of Atrial Myxoma. Histological analysis of the atrial myxoma was performed by both hematoxylin and eosin (H&E) staining and via immunofluorescent microscopic examination. H&E staining revealed that myxoma cells dispersed in myxoid matrix within myxomatous areas (Figure 1(a), Supplemental Figure 1 in Supplementary Material available online at http://dx.doi.org/10.1155/2016/2059584). Previous studies have revealed that the myxoma arises from a multipotential endocardial or subendocardial reserve mesenchymal cell which can differentiate into fibrocytes, myocytes, or endothelium [10]. To date, a number of putative markers for mesenchymal stem cells have been reported, including CD44 [11]. Our immunohistology data confirmed the expression of CD44 in myxoma. As a widely used cancer stem cell marker, CD44 expression indicates the malignant potential of cardiac myxoma [12]. It has been reported that myxomas are mainly composed of cellular elements including a variable number of blood cells [2] and amorphous myxoid matrix structures [13]. Moreover, cells in myxoid matrix rich lymphoma were more likely to express pan-B cell marker CD19 and the common leukocyte antigen CD45 [14]. And our further histology results confirmed the robust expression of CD45 and CD19 in myxoma (Figure 1(b), Supplemental Figure 1).

3.2. Isolation and Culturing of Myxoma Cells. Previous results revealed that myxoma cells express series transcription factors specific to phenotype of primitive cardiomyocytes and suggested that the development of cardiac myxoma originates from multipotential mesenchymal progenitors with a cardiomyogenic lineage [2]. We have described the isolation of cardiac stem cells that grow as phase-bright cells from murine hearts and similar methods were applied to isolate myxoma cells [9]. Explanted atrial myxoma was subjected to enzymatic digestion, and cultured phase-bright spherical cells that spontaneously separated from the myxoma samples were identified after 2 to 3 weeks (Figure 2(a)). These cells demonstrated a high nucleus-to-cytoplasm ratio (Figure 2(a), III). Flow cytometry analysis was performed to further characterize their cellular phenotypes and results revealed that this population of cells had higher expression of mesenchymal stem cell marker CD44 and pan-B cell marker CD19 and hematopoietic cell marker CD45. Cell proliferation analysis demonstrated linear growth with population doubling time

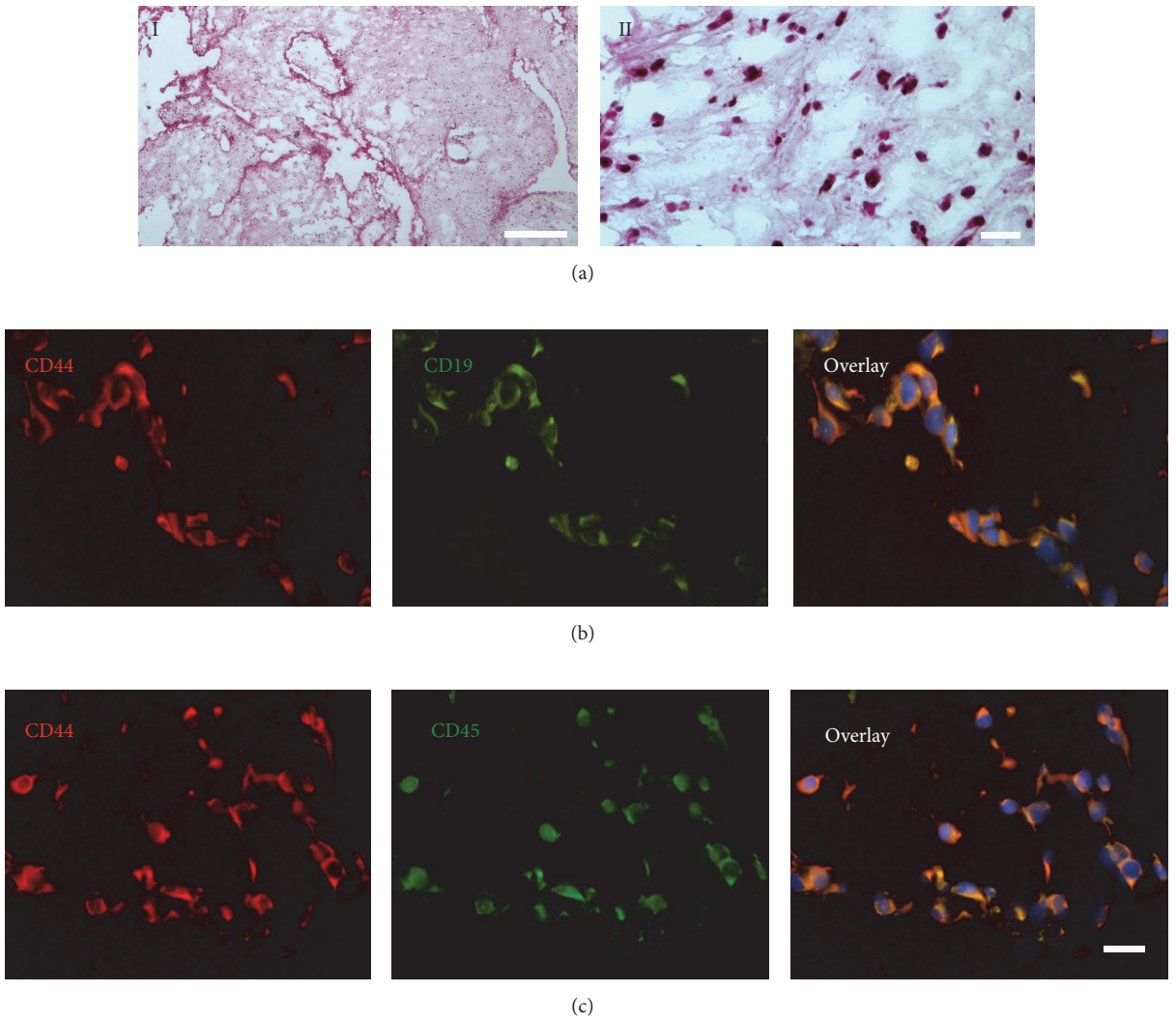

Figure 1: Phenotypic characterization of atrial myxoma. (a) Typical morphological features of myxoma by hematoxylin and eosin (HE) staining. Myxoma cells were dispersed within abundant myxoid matrix. Scale bar = 100 μm (I), =20 μm (II). (b) Myxoma cells were CD44 and CD19 double positive as confirmed by immunostaining. (c) Immunohistochemical characterization of myxomas revealed that myxoma cells were CD44 and CD45 double positive. Scale bar = 20 μm (b & c).

of approximately 7 days (data not shown). Similar results were obtained from all three samples.

3.3. In Vitro Differentiation of Myxoma Derived Cells. To investigate the multipotent differentiation of cardiac myxoma stem cells, we examined the differentiation of myxoma cells into various cell types. Similar to cardiac resident stem cells, these cells can differentiate into cardiomyocytes and smooth muscle cells, as documented by positive staining for cardiac troponin T (cTnT), connexin 43, myocyte enhancer factor 2C (MEF-2C), and α-smooth muscle actin (α-SMA) (Figure 3), similar to previous studies [1].

In this study, cardiac myxoma cells residing in a mucopolysaccharide-rich matrix were characterized in myxoma as a population of cells bearing the surface markers CD19, CD45, and CD44, which indicates these cells originating from hematopoietic cells with mesenchymal stem cell characterization. Moreover, we isolated myxoma cells with phase-bright culture method and confirmed that myxoma derived cells express robust level of CD44, CD19, and CD45. Furthermore, the pluripotency of this population cells also was verified by cardiomyocytes and smooth muscle cells differentiation in vitro.

Cancer stem cells are cancer cells that possess characteristics associated with normal stem cells, specifically the ability to give rise to heterogeneous cell populations in tumor [5]. It has been reported that tumor stem-like cells exist in benign tumors [15]. Important insights into cardiac myxoma tumorigenesis may come from studies of myxoma development and cell differentiation. Our results on identification of stem-like cells from myxoma suggested

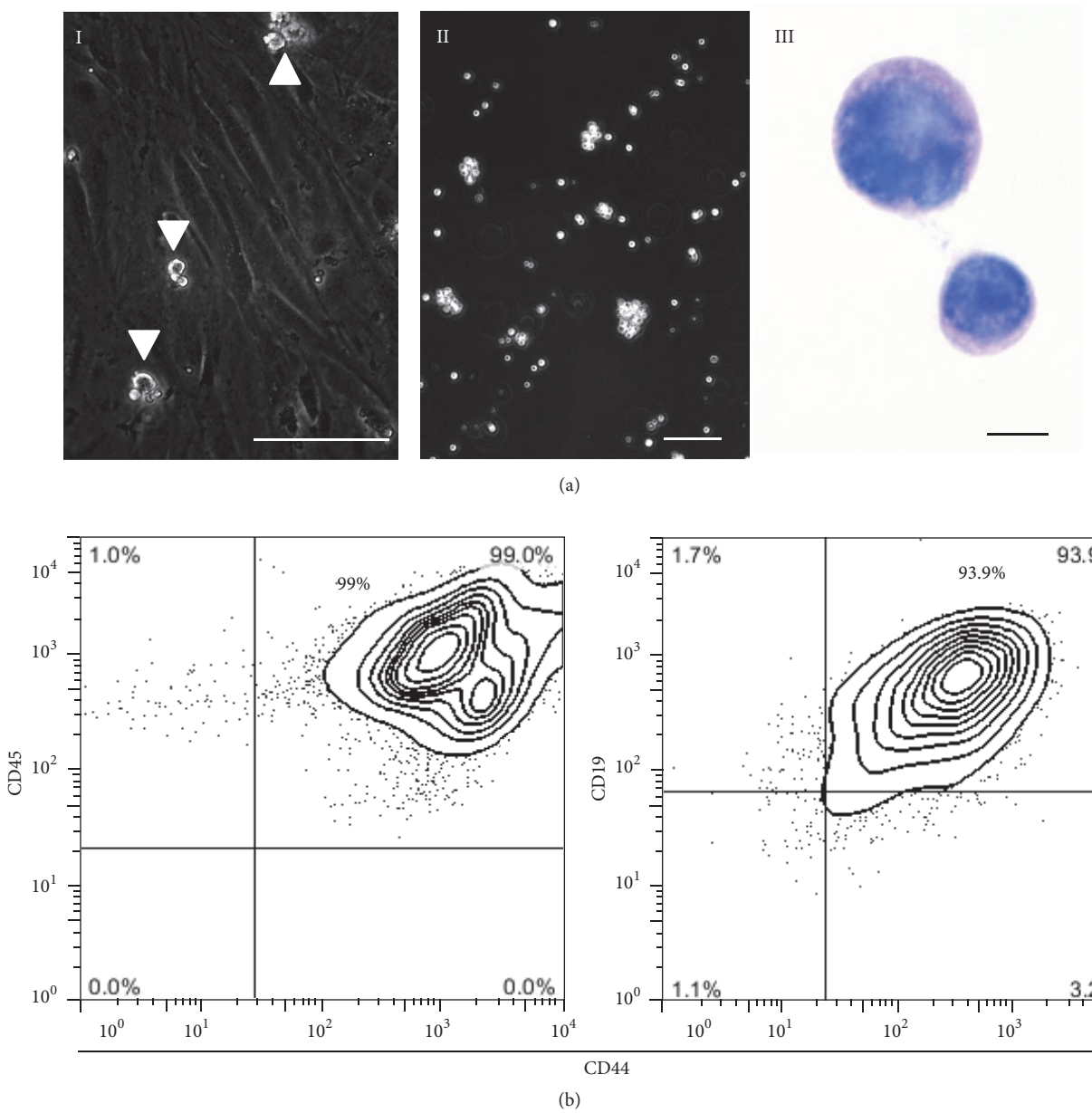

Figure 2: Characterization of subcultured myxoma cells from atrial myxoma. (a) After a period ranging from 1 to 3 weeks, phase-bright (arrow head) cells migrated over a layer of fibroblast-like cells (I, II). The phase-bright cells were collected, and Giemsa stain showed the cell with a large nucleus (III). Scale bar = 100 μm (I & II), =4 μm (III). (b) Quantification by FACS analysis of myxoma derived phase-bright cells. This cell population expresses robust CD44, CD19, and CD45.

that stem-like cells as tumor-initiating cells may be a general theme in these benign tumors.

In conclusion, our study is the first to isolate and phenotypically and functionally characterize cardiac myxoma stem-like cells by markers CD44, CD19, and CD45. In the meantime, we also verified their multipotent differentiation capabilities. This study may contribute to the elucidation of cardiac myxoma carcinogenetic mechanism and provides new insights for myxoma researches.

Competing Interests

The authors declare no competing financial interests.

Authors' Contributions

Xianghe Song and Danni Liu contributed equally to this work.

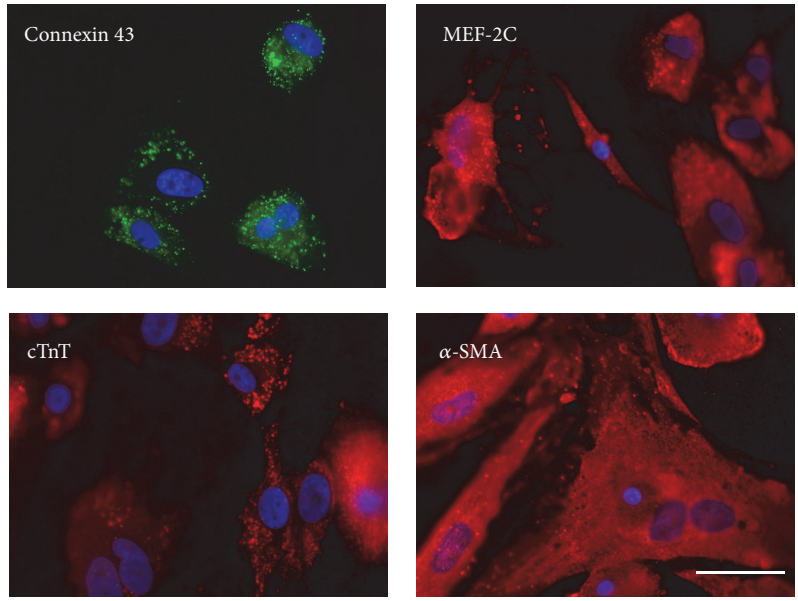

Figure 3: Multipotent capacity of myxoma derived cells. Immunostaining of subcultured myxoma derived phase-bright cells with connexin 43, myocyte enhancer factor 2C (MEF-2C), cardiac troponin T (cTnT), and α-smooth muscle actin (α-SMA). Scale bar = 10 μm.

Acknowledgments

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