

## STRO-1 positive pulmonary valve stem cells: preliminary report

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**Abstract:** Mesenchymal stem cells (MSCs) are defined by in vitro conditions which include their adherence to plastic. Stro-1 is one of the best known markers of mesenchymal stem cells (MSCs). The cardiac stem cells are known to reside in atrial and ventricular niches. However, valvular stem niches of the heart are often overlooked. Similarly, the expression of Stro-1 was not tested in cardiac valves. Thus we decided to perform a preliminary study on human samples to test whether or not in situ evidence supports the presence of a distinctive valvular stem niche. We used postmortem samples of pulmonary trunks and leaflets from donor cadavers (68-82 years old) without a cardiac cause of death. Within the pulmonary trunk wall the expression of Stro-1 was assessed in the vascular endothelial cells, including the vasa vasorum, vascular smooth muscle cells, and spindle-shaped adventitial and intimal cells. Moreover, a distinctive subpopulation of Stro-1+ cells was found both in the arterial walls and the pulmonary valve leaflets, displaying frequently mitotic morphologies. These were considered, based upon their antigenic phenotype, stem cells. Further studies should attempt to better individualize the valvular stem niche of the heart and to evaluate whether these stem cells could preserve, as in other tissues, a post mortem viable status, in order to be tested in vitro for their clonogenicity and phenotypes.

**Key Words:** cardiac stem cells, adult stem cells, mesenchymal stem cells, pulmonary valve, pulmonary trunk.

New heart valves may be engineered starting from mesenchymal stem cells (MSCs) [1]. Nevertheless, MSCs can be derived from either embryonic or adult tissues [2] but their features have been mostly characterised *in vitro*, as their adherence to plastic [3] is one of the dogmatic stem cell pre-requisites. Human bone marrow-derived MSCs are considered a suitable allogeneic source for tissue-engineered heart valves [4]. As heterogeneous subpopulations with

different degrees of stemness were identified among MSCs isolated by plastic adherence, the concept of MSC is difficult to be properly defined [5].

In order to establish more efficient treatments, we should gain a better understanding of the biological characteristics of heart stem cells [6]. The adult heart harbours a population of both stem and progenitor cells, which are able to replenish the population of cardiomyocytes, being thus adequate to better characterize

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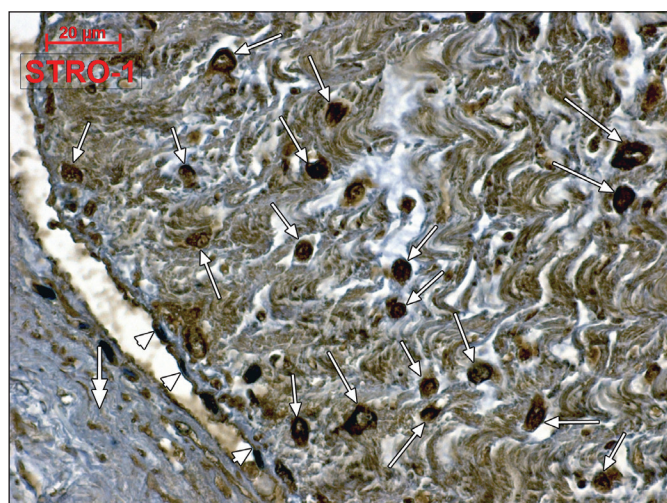
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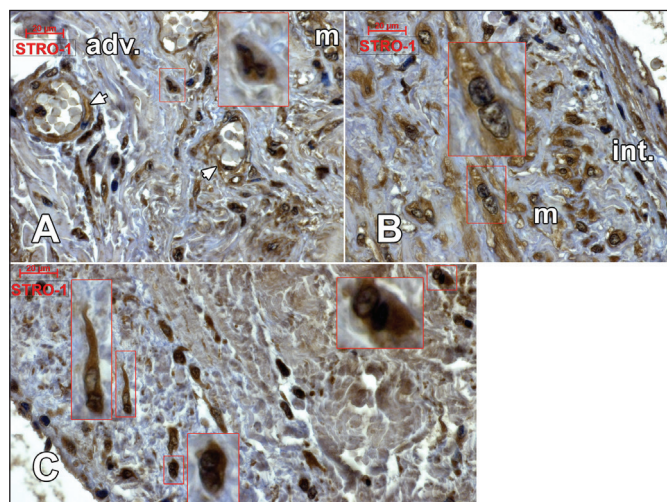
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the endogenous cardiac stem cells (CSCs) [7]. Clusters of CSCs were identified within the myocardium, leading to a classification of cardiac stem niches into atrial and ventricular, depending upon distinct stress levels of cardiac walls [8]. As far as we know, there aren't studies describing neither the presence, nor the phenotypical features of in situ CSCs of the human cardiac valves. Knowing the types and distribution of the stem cells might shed some light in cardiac pathogenesis and recovery after acute cardiovascular events, making it potentially useful in the analysis sudden cardiac death cases.



**Figure 1.** Human adult pulmonary valve of an aged donor (female, 73 years). Stro-1 positivity identifies valvular stem cells (arrows. Arrowheads indicate the endocardial sheath over the pulmonary semilunar leaflet. The double-headed arrow depicts the pulmonary trunk intima.



**Figure 2.** Human adult pulmonary valve of an aged donor (male, 82 years). The wall of the pulmonary trunk (A, B) and of the adjacent pulmonary semilunar leaflet (C) are consistent with the expression of Stro-1. In (A), Stro-1-positive vascular endothelial and periendothelial cells of the adventitial vasa vasorum of the pulmonary trunk are indicated by arrowheads. Possible mitosis of arterial and valvular Stro-1 cells are magnified in insets. adv, m, int: adventitia, media, intima of the pulmonary trunk.

Stro-1 is one of the most well known markers for MSCs [5, 9]. It may be involved in clonogenicity, and play a role in homing and angiogenesis of MSCs [5]. Expression of Stro-1 was reported in several tissues [5], but not in human adult cardiac valves. Therefore, we aimed to investigate the expression of Stro-1 in pulmonary semilunar valves and the adjacent pulmonary trunk wall in human aged specimens.

## MATERIALS AND METHOD

### Tissue samples

For this study we used human postmortem tissue (pulmonary trunks and valves), obtained during the autopsy of ten cadavers (with a 6:4 sex ratio) with ages between 68 and 82 years. The study was conducted according to the national laws regarding the use of cadaveric material for research purposes (including Law 104/2003 regarding the manipulation of human cadavers, and the Ordonance 1/2001 regarding the functioning of the legal medicine services, with all subsequent and adjacent regulations, as well as the general principles from the Declaration of Helsinki, Cairo revision.

### Antibodies

The primary antibody used in the study was anti-Stro-1 (clone STRO-1, Sigma-Aldrich, Sigma-Aldrich Co., MO, USA, 1:100).

### Immunohistochemistry

Tissue samples were fixed for 24 hours in buffered formalin (8%), oriented anatomically and processed with an automatic histoprocessor (Diapath, Martinengo, BG, Italy) with paraffin embedding. Sections were cut manually at 3 µm and mounted on SuperFrost® electrostatic slides for immunohistochemistry (Thermo Scientific, Menzel-Gläser, Braunschweig, Germany). Histological evaluations used 3 µm thick sections stained with hematoxylin and eosin.

The sections were deparaffinized in "Slide bright" and a underwent a descending series of alcohol rinses, and then rehydrated in distilled water. Endogenous peroxidase was blocked with 3% H<sub>2</sub>O<sub>2</sub> for 5 min at room temperature. For protein blocking, the sections were incubated for 5-10 min at room temperature with a background sniper (Biocare Medical, Concord, CA, USA). Then, the sections were incubated with the primary antibodies for 30 min at room temperature, followed by 30 min incubation with a polymer at the same temperature (MACH 4 detection system, Biocare Medical, Concord, CA). The next steps consisted of incubation for 5 min at room temperature with 3,3'-Diaminobenzidine (DAB, Biocare Medical, Concord, CA) and counterstaining with hematoxylin. Sections without primary antibodies were considered as internal negative controls.

The microscopic slides were analyzed and

micrographs were captured and scaled using a Zeiss working station, previously described [10].

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## RESULTS

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On the slides, we accurately identified the pulmonary trunk wall and the adjacent pulmonary semilunar leaflets. The arterial wall consisted, centripetally, of an adventitia covered by subepicardial fat, media and intima covering the endothelial cells. The pulmonary leaflets were rich in collagen fibers. We found numerous cells embedded in the collagen matrix, most of them round in section, expressing Stro-1 (Fig. 1) which were evaluated according to the marker specificity as valvular stem cells (VSCs).

Within the pulmonary trunk wall, we found Stro-1-positive cells in the adventitia and intima, as well as Stro-1-positive endothelial and periendothelial cells, belonging to the adventitial vasa vasorum (Fig. 2) or to the periadventitial fat-embedded vessels. Expression of Stro-1 was also located in the vascular smooth muscle cells of the pulmonary trunk media. Noteworthy, the pulmonary trunk layers, as well as the pulmonary valve leaflets, were populated by cells expressing Stro-1, which were histologically positive for mitotic activities (Fig. 2), thus being considered cycling stem cells.

The negative controls backed up the results, since the slides without the primary antibody did not yield any positive labeling.

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## DISCUSSION

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The stem cell niches were recently classified anatomically into specialized (few cell types lying on the basement epithelial membranes) and nonspecialized (various cell types distributed within mesenchymal tissues) [11]. According to that concept, the niches we explored in the pulmonary trunk wall and the pulmonary valve leaflets are nonspecialized niches containing MSCs, vascular endothelial cells and stromal spindle-shaped cells.

Vessel-wall spindle-shaped MSCs (vw-MSCs) were found expressing CD44, CD90, CD105, like bone marrow-derived MSCs, as well as stemness markers, such as Stro-1, Notch-1 and OCT4 [12]. These vw-MSCs, as well as bone marrow-derived MSCs, might play a role in the pathogenesis of atherosclerosis [12-15]. Moreover, resident vw-MSCs may enter the circulation to enrich the population of circulating progenitor/stem cells that are able to engraft other tissues [16]. A limitation of Stro-

1 labeling is that it cannot distinguish between intrinsic and extrinsic MSCs. Use of additional markers might not exclude an extrinsic origin of MSCs, as these could have resided in the tissue long enough to have lost the epitopes of the original cell lineages, as was previously discussed on the CSCs [17].

The bone marrow cells that express Stro-1 are capable of differentiating into multiple mesenchymal lineages including: (a) hematopoiesis-supportive stromal cells with a vascular smooth muscle-like phenotype, (b) adipocytes, (c) chondrocytes and (d) osteoblasts [18]. Similarly to our study on human tissue, the endothelial and perivascular expression of Stro-1 was assessed in various tissues in rats [9, 19]. The reason of this specificity was investigated and it was reached the conclusion that Stro-1 is intrinsically a 75 kD endothelial antigen, and the expression of Stro-1 in MSCs could be an induced event [9]. It is so reinforced the issue raised by Ning *et al.* (2011) who commented that “The question is, other than being an MSC marker and an endothelial antigen, what is Stro-1?” [9].

Although a hematopoietic origin of cardiac valve stem cells was assessed experimentally, there were no morphological differences found between donor and host valve interstitial cells [20]. Our findings support the concept that the origin of adult valve interstitial cells, such as fibroblasts, myofibroblasts and smooth muscle cells is not exclusively related to developmental sources [20]. Moreover, it cannot be ruled out that resident VSCs populate the adult pulmonary valve leaflets, in addition to a bone marrow MSCs supply. Beyond the origin of VSCs, a cardiac valvular niche could be distinguished from the myocardial, atrial and ventricular, cardiac stem cell niches, and should be further evaluated to establish the pathogenic events which alter a physiologic process of valvular regeneration into a lesion onset.

We didn't find previous studies assessing the in situ Stro-1 expression in human valves, although various experiments were performed to evaluate various autologous donor tissues for valvular engineering, such as bone marrow or umbilical cord [1, 21]. Further studies should evaluate whether stem cells of the valvular niche could preserve, such as in other tissues [22], a post mortem viable status, in order to be tested *in vitro* for their clonogenicity and phenotypes.

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**Conflict of interests.** None.

## References

1. Sutherland FW, Perry TE, Yu Y *et al.* From stem cells to viable autologous semilunar heart valve. *Circulation* 2005; 111: 2783-2791.
2. Zhang Y, Liang X, Lian Q *et al.* Perspective and challenges of mesenchymal stem cells for cardiovascular regeneration. *Expert review of cardiovascular therapy* 2013; 11: 505-517.



3. Dominici M, Le Blanc K, Mueller I *et al.* Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006; 8: 315-317.
4. Batten P, Sarathchandra P, Antoniow JW *et al.* Human mesenchymal stem cells induce T cell anergy and downregulate T cell allo-responses via the TH2 pathway: relevance to tissue engineering human heart valves. *Tissue engineering* 2006; 12: 2263-2273.
5. Lv FJ, Tuan RS, Cheung KM *et al.* The surface markers and identity of human mesenchymal stem cells. *Stem Cells* 2014, DOI: 10.1002/stem.1681.
6. Kawaguchi N. Adult cardiac-derived stem cells: differentiation and survival regulators. *Vitamins and hormones* 2011; 87: 111-125.
7. Torella D, Ellison GM, Nadal-Ginard B *et al.* Cardiac stem and progenitor cell biology for regenerative medicine. *Trends in cardiovascular medicine* 2005; 15: 229-236.
8. Leri A, Kajstura J, Anversa P. Cardiac stem cells and mechanisms of myocardial regeneration. *Physiological reviews* 2005; 85: 1373-1416.
9. Ning H, Lin G, Lue TF *et al.* Mesenchymal stem cell marker Stro-1 is a 75 kd endothelial antigen. *Biochemical and biophysical research communications* 2011; 413: 353-357.
10. Rusu MC, Jianu AM, Pop F *et al.* Immunolocalization of 200 kDa neurofilaments in human cardiac endothelial cells. *Acta Histochemica* 2012; 114: 842-845.
11. Ema H, Suda T. Two anatomically distinct niches regulate stem cell activity. *Blood* 2012; 120: 2174-2181.
12. Vasuri F, Fittipaldi S, Pasquinelli G. Arterial calcification: Finger-pointing at resident and circulating stem cells. *World journal of stem cells* 2014; 6: 540-551.
13. Wang ZX, Wang CQ, Li XY *et al.* Mesenchymal stem cells alleviate atherosclerosis by elevating number and function of CD4CD25 FOXP3 regulatory T-cells and inhibiting macrophage foam cell formation. *Molecular and cellular biochemistry* 2014, DOI: 10.1007/s11010-014-2272-2273.
14. Lin YL, Yet SF, Hsu YT *et al.* Mesenchymal Stem Cells Ameliorate Atherosclerotic Lesions via Restoring Endothelial Function. *Stem cells translational medicine* 2014, DOI: 10.5966/sctm.2014-0091.
15. Yan J, Tie G, Xu TY *et al.* Mesenchymal stem cells as a treatment for peripheral arterial disease: current status and potential impact of type II diabetes on their therapeutic efficacy. *Stem cell reviews* 2013; 9: 360-372.
16. Abedin M, Tintut Y, Demer LL. Mesenchymal stem cells and the artery wall. *Circulation research* 2004; 95: 671-676.
17. Beltrami AP, Barlucchi L, Torella D *et al.* Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell* 2003; 114: 763-776.
18. Dennis JE, Carbillet JB, Caplan AI *et al.* The STRO-1+ marrow cell population is multipotential. *Cells, tissues, organs* 2002; 170: 73-82.
19. Lin G, Liu G, Banie L *et al.* Tissue distribution of mesenchymal stem cell marker Stro-1. *Stem cells and development* 2011; 20: 1747-1752.
20. Visconti RP, Ebihara Y, LaRue AC *et al.* An in vivo analysis of hematopoietic stem cell potential: hematopoietic origin of cardiac valve interstitial cells. *Circulation research* 2006; 98: 690-696.
21. Schmidt D, Mol A, Odermatt B *et al.* Engineering of biologically active living heart valve leaflets using human umbilical cord-derived progenitor cells. *Tissue engineering* 2006; 12: 3223-3232.
22. Latil M, Rocheteau P, Chatre L *et al.* Skeletal muscle stem cells adopt a dormant cell state post mortem and retain regenerative capacity. *Nature communications* 2012; 3: 903.