

Telocytes in human epicardium

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Abstract

The existence of the epicardial telocytes was previously documented by immunohistochemistry (IHC) or immunofluorescence. We have also demonstrated recently that telocytes are present in mice epicardium, within the cardiac stem-cell niches, and, possibly, they are acting as nurse cells for the cardiomyocyte progenitors. The rationale of this study was to show that telocytes do exist in human (sub)epicardium, too. Human autopsy hearts from 10 adults and 15 fetuses were used for conventional IHC for c-kit/CD117, CD34, vimentin, S-100, τ , Neurokinin 1, as well as using laser confocal microscopy. Tissue samples obtained by surgical biopsies from 10 adults were studied by digital transmission electron microscopy (TEM). Double immunolabelling for c-kit/CD34 and, for c-kit/vimentin suggests that in human beings, epicardial telocytes share similar immunophenotype features with myocardial telocytes. The presence of the telocytes in human epicardium is shown by TEM. Epicardial telocytes, like any of the telocytes are defined by telopodes, their cell prolongations, which are very long (several tens of μm), very thin (0.1–0.2 μm , below the resolving power of light microscopy) and with moniliform configuration. The interconnected epicardial telocytes create a 3D cellular network, connected with the 3D network of myocardial telocytes. TEM documented that telocytes release shed microvesicles or exocytotic multivesicular bodies in the intercellular space. The human epicardial telocytes have similar phenotype (TEM and IHC) with telocytes located among human working cardiomyocyte. It remains to be established the role(s) of telocytes in cardiac renewing/repair/regeneration processes, and also the pathological aspects induced by their 'functional inhibition', or by their variation in number. We consider telocytes as a real candidate for future developments of autologous cell-based therapy in heart diseases.

Keywords: telocytes • telopodes • epicardium • c-kit/CD117 • shed vesicles • exosomes • cardiac repair • myocardial regeneration • cardiac progenitors • cardiac niches • stem cells

Introduction

Frequently over-passed and considered to be only a bare serosal cover for the myocardial pump, nowadays the epicardium is revealing some of its cellular and molecular secrets [1]. Recent studies documented the possible key role of the epicardial cells in myocardial development [2–12] and in cardiac regeneration and repair, eventually [13–33]. However, insufficient attention was paid to fully characterize all cell types populating the epicardium. We previously found by serendipity within human and mammalian myocardium

the presence of a distinct cell population – telocytes [34]. We coined the term 'telocyte', which seems internationally accepted, replacing the former one: interstitial Cajal-like cells; acronym ICLC – that we used during the last 4–5 years [35–44]. The most characteristic feature of telocytes is their very long and very thin prolongations, which we named telopodes [34]. Several groups also demonstrated the presence of c-kit⁺ cells within epicardium [45–48], contributing to cardiac cell turnover [46, 47], but the identity of these cells

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remained unknown. Here we show by transmission electron microscopy (TEM) (seeing is believing!) and immunofluorescence the presence of telocytes and telopodes in human beings. Their functional significance in epicardium remains to be explored.

Material and methods

Tissue specimens

For immunohistochemistry (IHC), cardiac tissue used in the study was obtained either from autopsy or from archived (paraffin embedded) material. We included cardiac samples from five individuals who died of non-cardiac diseases aged 55–72 years, as well as from 15 fetuses aged 17 to 23 weeks. For confocal microscopy, left ventricular myocardial tissues were obtained from three donor hearts that for technical reasons were not used for heart transplantation. This study was approved by the Ethics Committee. For electron microscopy, human right atrial appendage tissue was obtained from 10 patients undergoing coronary artery bypass grafting or mitral surgery. Tissue samples were collected from patients who had given informed consent before surgery at the 'C.C. Iliescu' Institute of Cardiovascular Diseases, Bucharest.

Confocal microscopy

The specimens were fixed for 4 hours in 4% freshly prepared paraformaldehyde, cryoprotected with 20% sucrose, quick-frozen in methylbutane at -130°C and stored at -80°C . Frozen sections $20\ \mu\text{m}$ thick were placed on gelatin-coated slides and then microwave-processed in citrate buffer, pH 6.0. After repeated washes in PBS, the tissue sections were blocked and permeabilized with 100 mM glycine, 0.1% carboxylated bovine serum albumin and 0.01% Triton X-100 in PBS, pH 7.6. The cryosections were incubated overnight with the primary polyclonal antibody against c-kit (Dako, Glostrup, Denmark). After washing in PBS, the preparations were incubated with biotinylated donkey anti-rabbit IgG followed by streptavidin-Cy-2 (Biotrend, Cologne, Germany). The nuclei were stained with 0.001% DAPI (Molecular Probes, Carlsbad, CA, USA) and myofibrils were stained with phalloidin conjugated with TRITC (Sigma). Omission of the primary antibody served as a negative control.

Immunohistochemistry

Sections taken from epicardium to myocardium were fixed in 10% formalin and paraffin embedded. Sequential 3–5 μm sections were stained with haematoxylin and eosin for histological evaluation. Simple immunostaining was performed with a Dako EnvisionTM + Dual Link System-HRP (Dako, Carpinteria, CA, USA), according to the manufacturer's instructions, and counterstained with haematoxylin. We determined on serial sections the expression of: CD117 (polyclonal, Dako, 1:250), vimentin (clone V9, Dako, 1:50), CD34 (clone QBend 10, Dako, 1:50), S100 (polyclonal rabbit, Dako, Glostrup Denmark, 1:400), Nestin (clone 10c2, Santa Cruz Biotechnology, Santa Cruz, CA, USA 1:500) and CD57 (clone TB01 Dako, 1:50). Sections

were deparaffinized, rehydrated and rinsed in phosphate-buffered solution at pH 7.4. Retrieval by cooking in specific buffer was completed in water bath, 40 min. at 96°C for vimentin, CD117 and S100. Appropriate endogenous blocking peroxidase was completed before CD34, Nestin and CD57. Double immunostaining was performed with PicTure – Double staining kit (ZYMED Laboratories, S. San Francisco, CA, USA), using two enzyme polymer conjugates (Gt antimouse IgG-HRP and Gt anti-rabbit IgG-AP) each coupled with one chromogen (3,3-Diaminobenzidine and Fast – Red), for simultaneous double antigen detection. Double stainings were carried out for CD117 together with CD34, and CD117 with vimentin. Sections incubated with non-immune serum were used as negative controls. Dual stained slides were cover slipped without counterstaining. The intensity of positivity for markers tested was assessed semi quantitatively using the Quick score method, which takes into account intensity and distribution of positivity.

Transmission electron microscopy

Small fragments of myocardium with epicardium were processed for TEM according to routine procedures, as we previously described [33, 36–40]. Light microscopy was performed on about 1 μm semithin sections stained with 1% toluidine blue and digital images were recorded using a charge-coupled device (CCD) Axiocam HRc Zeiss camera with AxioVision software (Carl Zeiss Imaging solution GmbH, Oberkochen, Germany), on Nikon Eclipse E600 microscope (Nikon Instruments Inc., Tokyo, Japan). Ultrathin sections stained with uranyl acetate and Reynolds's lead citrate solutions were examined using a Morgagni 286 TEM (FEI Company, Eindhoven, The Netherlands) at 60 kV. Digital electron micrographs were recorded with a MegaView III CCD using iTEM-SIS software (Olympus, Soft Imaging System GmbH, Münster, Germany). Digital coloured TEM images were processed using Adobe Photoshop software (Adobe Systems, Inc., San Jose, CA, USA). The colour codes used to emphasize different types of cells are blue for telocytes, brown for cardiomyocyte, yellow for adipocytes and green for nerve fibres.

Results

Immunofluorescence correlated with TEM

Figure 1A shows by confocal microscopy c-kit⁺ cells in human subepicardial area. Figure 1B presents a higher magnification view. The epicardial localization of telocytes beneath the mesothelial cells is proven by TEM (Fig. 1C).

Immunohistochemistry of epicardium

Serial sections from human epicardium were tested by IHC for antigens usually expressed by telocytes in other tissues: c-kit, CD34, S-100, vimentin, τ . Sections containing archetypal interstitial cell of Cajal (ICC) from the digestive tract were used as positive controls (results not shown in this study). c-kit cell positivity was observed in epicardium, by cells having prolongations with an apparently random distribution, without significant difference in

staining intensity, between epicardial sides (Fig. 2A–C). c-kit positivity of some epicardial cells was already shown [41, 45–48].

High levels of CD34 positivity were noted in the epicardium, but due to the vicinity of a small vessels network, the discrimination between telocytes and endothelial cells was sometimes difficult. Telocytes morphology (peculiar telopodes) may be mimicked by the contiguous aspect of endothelial layer in different incidences (Fig. 3A). In order to individualize the CD34 signal from telocytes from that of endothelial cells, double labelling c-kit/CD34 was performed (Fig. 3B). Similarly, double labelling c-kit/vimentin is shown in Fig. 3C. The aspect reveals the telocytes location in the close vicinity of a capillary, an aspect similar with that previously observed in myocardium [36–38, 41]. Figure 4A shows telocytes positive for S-100. Immunolabelling for τ protein was similar with that for S-100 (Fig. 3D) Immunolabelling for CD57 (NK1) was positive for telocytes. However, the results are difficult to interpret because, apparently, the reaction was less specific in cells with prolongations (Fig. 4B).

Transmission electron microscopy

Electron microscopy study unequivocally demonstrated the presence of the telocytes in human epicardium, having their distinctive ultrastructural features according to the TEM diagnostic criteria for telocytes, as described in myocardium [30, 31, 34, 36–40] and other organs [e.g. 35]. Figures 5–7 present distinctive ultrastructural details of telocytes. In order to make the telocytes more evident, images were digitally coloured in blue. In Fig. 5A it appears in the cell profile of an epicardial telocyte, in between collagen fascicles, in the neighbourhood of a cardiomyocyte.

The distinctive characteristics of telopodes are shown in Fig. 5A and B. Figure 5A shows a 2-telopode telocyte. Of

course, the number of prolongations as well as the ramifications of telopodes depend on the site and angle of section, because TEM is essentially a 2D examination of an extremely thin section (~60 nm).

The telopodes shown in Fig. 5A have a length of about 25 and 20 mM, respectively. The calibre of telopodes is uneven, mostly below 0.2 mM. The aspect of telopodes is moniliform, with a dichotomous pattern of branching. Figure 5B presents, at a lower magnification, two very long (about 50 mM) and extremely thin (about 100 nm) telopodes, one with very convoluted profile (the cell process lying over the cardiomyocyte) and another having a surface without flexions or irregularities. For the last one the close vicinity with elastic fibres deserves to be mentioned. Figure 6 is illustrating the exchange of material between telocytes and the extracellular matrix or other cells in subepicardium. The labyrinthine shape of the telopodes seems to help this telocyte to deliver either single vesicles, the so-called ‘shed vesicles’ (Fig. 6A), or multivesicular bodies, as exosomes (Fig. 6B and C). Figure 6B clearly shows the close vicinity (under 0.5 mM) of a nerve fibre with the telocyte and its telopode; this was repeatedly presented before as a diagnostic criterion for TEM identification of telocytes (as well as the vicinity with capillaries) [34, 37–40].

Figure 7 was digitally coloured, in order to differentially label myocytes (Sienna brown), nerve fibres (green) and telocytes (blue). Figure 7A is a typical aspect in epicardium, which presents relationships of a telopode with a large nerve bundle. Figure 7C reveals another typical aspect about telocytes: the frequent positioning at the ‘meeting point’ of different cell types composing cardiac tissue – myocytes, nerve fibres and adipocytes. The telopodes may establish close contacts with other interstitial cell (Fig. 7B and C), previously named ‘stromal synapses’ [49].

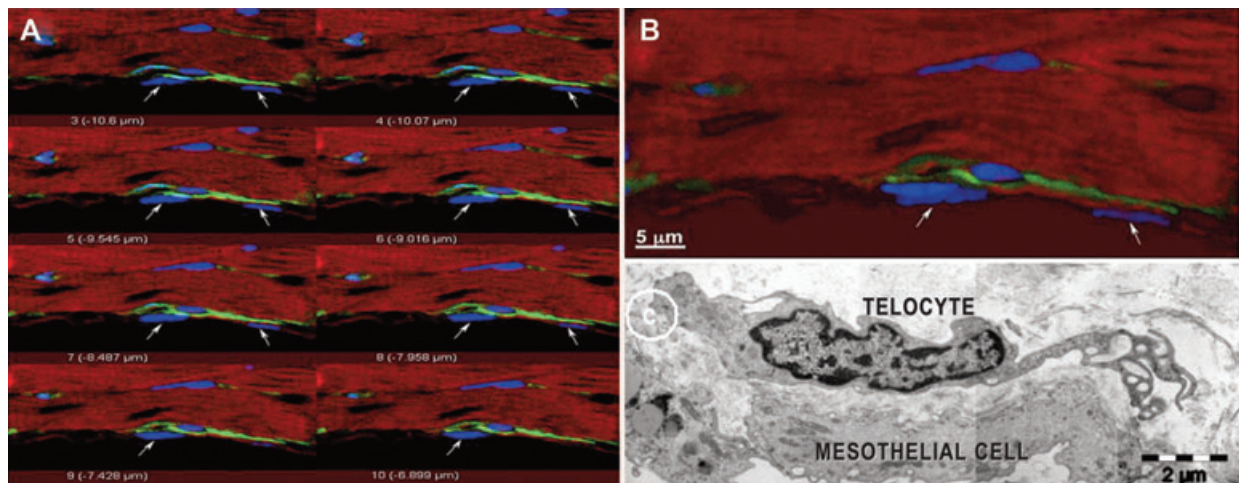


Fig. 1 (A and B) Confocal microscopy. c-kit⁺ cells in human subepicardial area. (A). Gallery of LSM (laser scanning microscopy) images obtained at 0.529 μm steps showing the spatial distribution of c-kit⁺ cells with long processes under mesothelial cells (arrows) in human myocardium. (B) Maximum projection of LSM images shown in (A). Mesothelial cells are indicated with arrows. (C) TEM: one telocyte is located near the mesothelial cells of the same human epicardium.

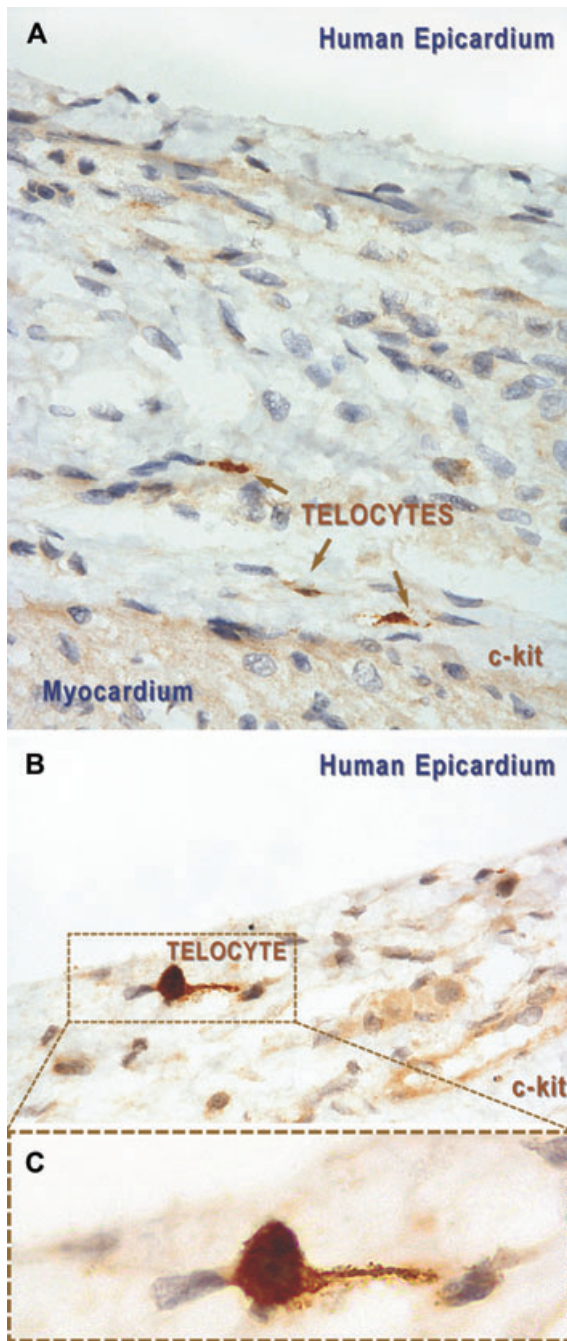


Fig. 2 Immunostaining for c-kit in human epicardium. (A) c-kit positivity of telocytes is more evident at the level of the cell bodies in the deeper epicardial layer. Original magnification, 40 \times . (B) Immunostaining revealing a c-kit⁺ cell near epicardial surface. Note that immunoreactivity is not restricted to the cell body, and clearly reveals the emergence of telopodes. Original magnification, 60 \times . (C) Higher magnification of the telocytes presented in Fig. 2B. Telopodes are running out in opposite directions and their thickness at the advent is rather discrete. The staining is concomitant on cell body and cell prolongations. Original magnification, 100 \times .

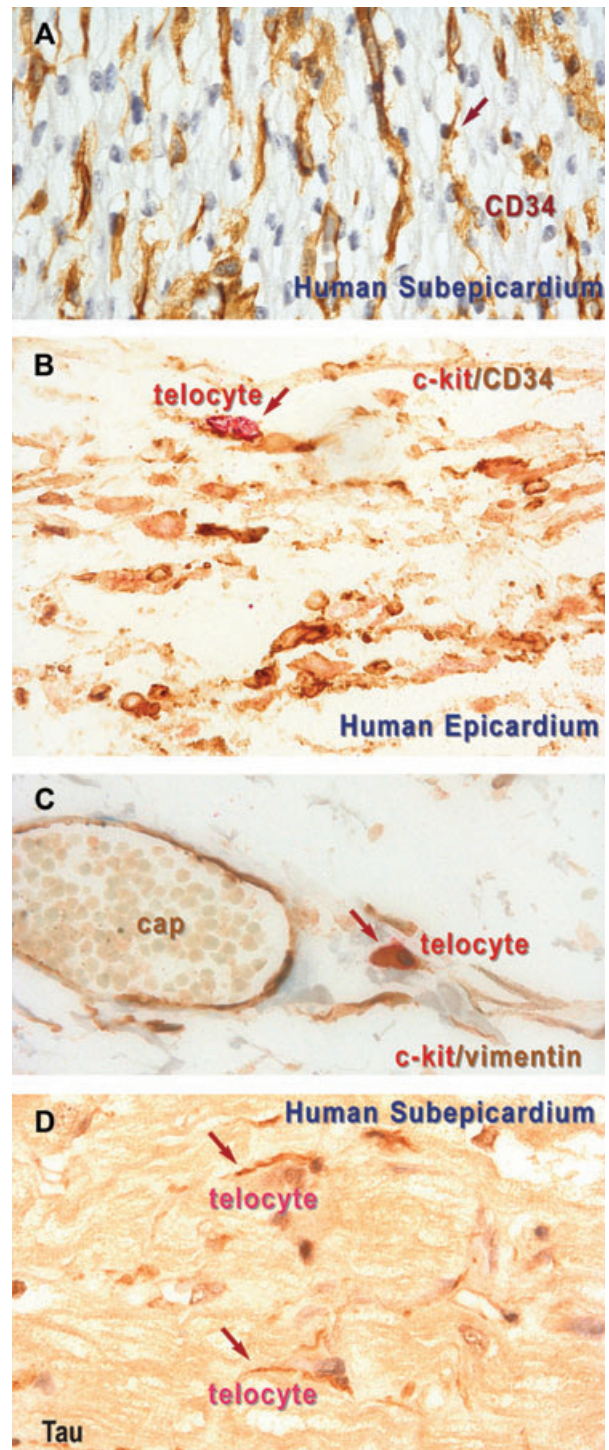


Fig. 3 (A) Immunostaining for CD34 (brown). CD34 positivity might be expressed by telocytes and endothelial cells. (B) Localization of double positive cells for c-Kit (red) and CD34 (brown). (C) Human epicardium, deep layer. Vimentin positivity is stronger than c-Kit positivity by sandwich method. (D) Cells expressing τ protein (arrows). (A–D) Original magnification, 40 \times .

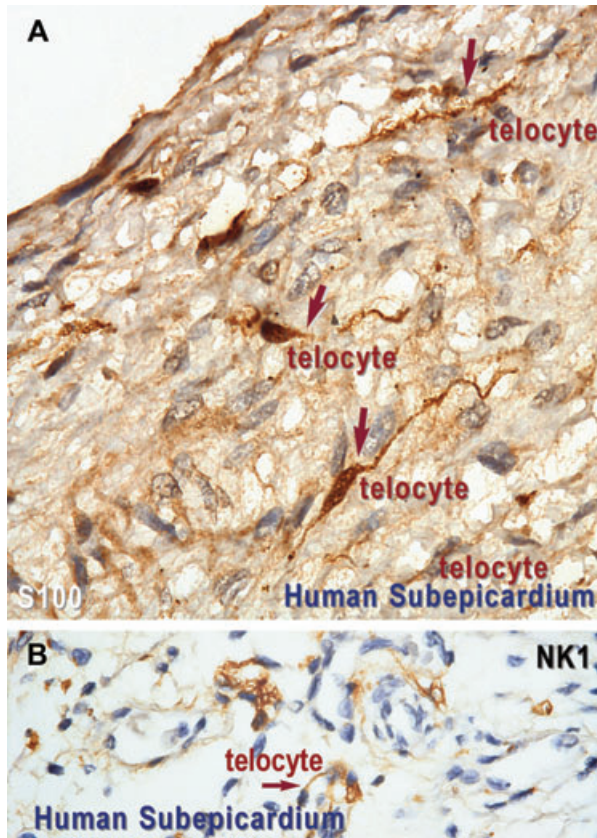


Fig. 4 (A) reveals a cluster of three cells positive for S-100 (arrows) distributed in human subepicardium. (B) Immunostaining for CD57 (NK1) in human subepicardium. Thin cellular processes are stained in the vicinity of capillaries. Original magnification, 40 \times .

Discussion

The cells identified as telocytes in human epicardium meet the characteristic ultrastructural criteria already established for telocyte:

- (1) Location in the connective interstitium, in the extraepithelial space, among functional elements (*e.g.* nerve endings, blood capillaries, muscle cells, immunoreactive cells) – Figs 5A and B, 6B, 7A–C and
- (2) Telopodes: (i) typical long, thin, moniliform prolongations, with; (ii) dichotomic branching pattern (Figs 5A and B, 6A); (iii) specialized cell-to-cell junctions (*e.g.* ‘stromal synapses’ [49]; (iv) attachment plaques connect the telocytes to the extracellular matrix and the perivascular elastic material (Fig. 5A and B). The (sub)epicardial telocytes apparently make a mesh-like network, comparable with a fishing net woven by a ‘short-sighted fisherman’. Similar data were previously reported for telocytes in myocardial sleeves of human pulmonary veins [50], as well as in mice epicardium [30, 31,

33, 41]. Anyway, epicardial telopodes appear very similar with telopodes from myocardium [36–40, 41] and other organs [50–55].

The screening of the literature revealed few studies evaluating c-kit immunoreactivity in the epicardium, and most of them have focused on the role of c-kit in cardiac development or repair [46–48]. Our data emphasize that IHC alone is not enough for the positive diagnosis of telocytes. However, it remains a useful tool for semi-quantitative data analysis, and evaluation of co-expression of antigens. As a consequence, the expression of c-kit alone is not sufficient to characterize an epicardial cell as telocyte. Co-expression of a panel of other markers, including CD34, vimentin, nestin, S-100 might be indicative for the telocytic nature of an interstitial c-kit⁺ cell.

A striking feature of epicardial telocytes is the releasing of shed microvesicles or multivesicular bodies as exosomes in the extracellular matrix (Fig. 6A–C). The nature and the functional meaning of material delivered (and processed?) by these microvesicles remain to be established. Of note, recent studies [57–59] have reported that microvesicles transfer tissue specific RNAs and proteins and this newly recognized system of intercellular communications might have a critical role in physiological and pathological processes and offer a new research direction for stem cell therapy [60–63]. One may speculate that such ‘cargo’ might be exploited as a therapeutic tool, as we have recently suggested [30].

In our study there were no significant differences of immunohistochemical profile in human beings, when tissue specimens from adults were compared with tissue specimens of foetal origin. Data presented here correlate well with studies approaching epicardial telocytes with similar or complementary methodologies (immunofluorescence or TEM), in human beings and/or murine species [30, 31, 33, 41]. These results should be correlated with the fact that myocardium was explored much more extensively than epicardium, in both normal and pathological conditions. It is interesting to observe that even in studies centred on ultrastructural analysis of transmural biopsy specimens (during ischemia-reperfusion injury studies) and ‘no attempts were made to analyse endocardium and epicardium separately’ [60].

A tempting idea is that this cell might have a therapeutic potential for the design of future cell-based cardiac repair strategies. At least apparently, ‘the usual treatment’ using mesenchymal stem cells (MSC) is not very encouraging: most recent data provided by Hu’s group [64] suggest that only 3–4% of injected MSCs are remaining in the heart. It would be also very interesting to see what happens in a heart when a portion of epicardium is lacking, as well as in extreme situation of pericardiectomy [61, 62]. Also, cell turnover should be monitored after protocols intending to transform pericardium in biological scaffolds during tissue engineering procedures (decellularized allografts after treatment with SDS) [63]. It also remains to be established if telocytes might be acknowledged as major a player in mesothelial-cell-induced cardiac repair.

In conclusion, at least for the moment, we strongly believe in our previous hypothesis that telocytes could be either tracks or are nursing cardiac progenitor cells for regeneration. It is attractive to

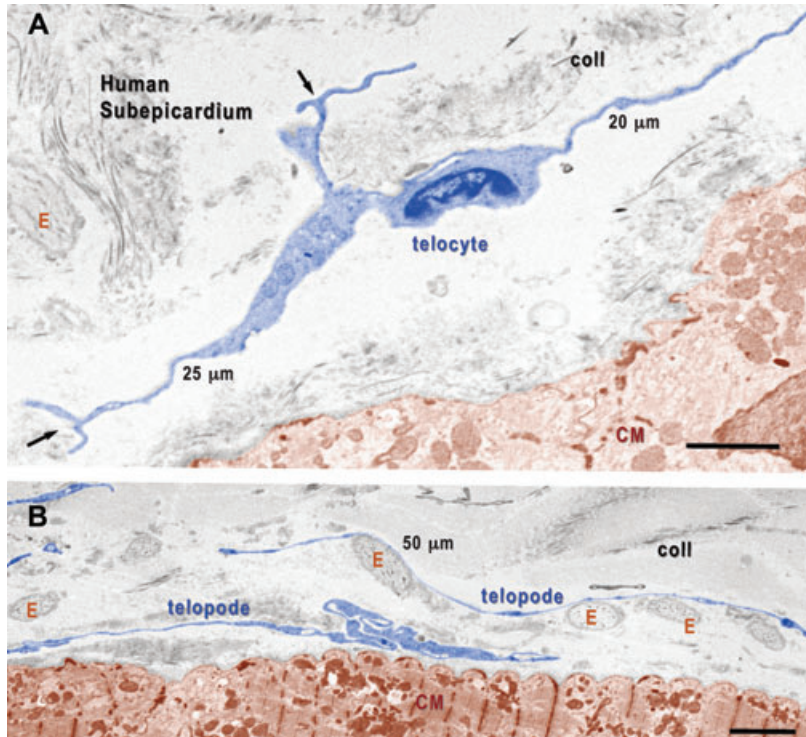


Fig. 5 Digital coloured TEM images show telocytes (blue) in the human subepicardium, bordering the peripheral cardiomyocytes (CM, highlighted in brown). (A) TEM image of a 20 µm long telocyte with three telopodes, illustrating the distinctive dichotomous pattern of branching (arrows). (B) The thin, moniliform telopodes (length for the convoluted telopode ~30 µm, upper telopode ~38 µm) overlap each other in the periphery of myocardium creating a separation leaf. E-elastic fibres, coll-collagen fibres. Scale bar ~5µm.

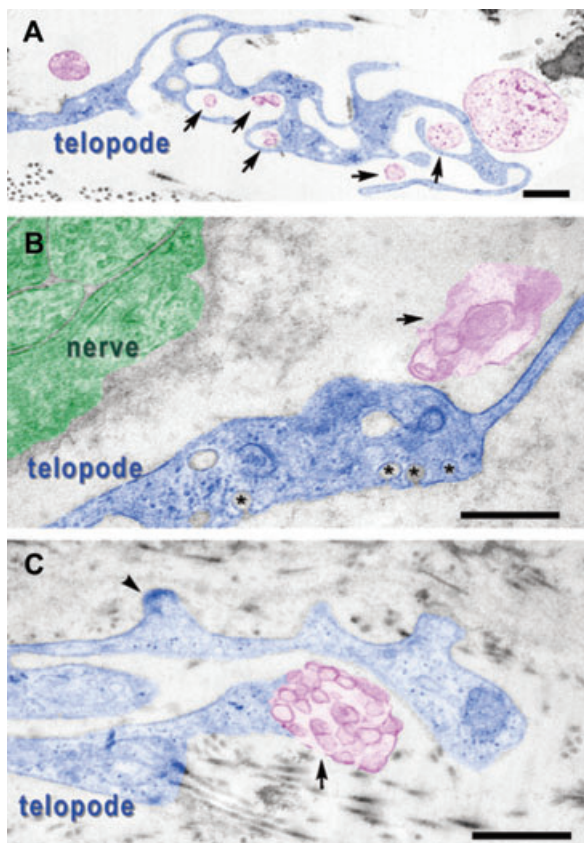
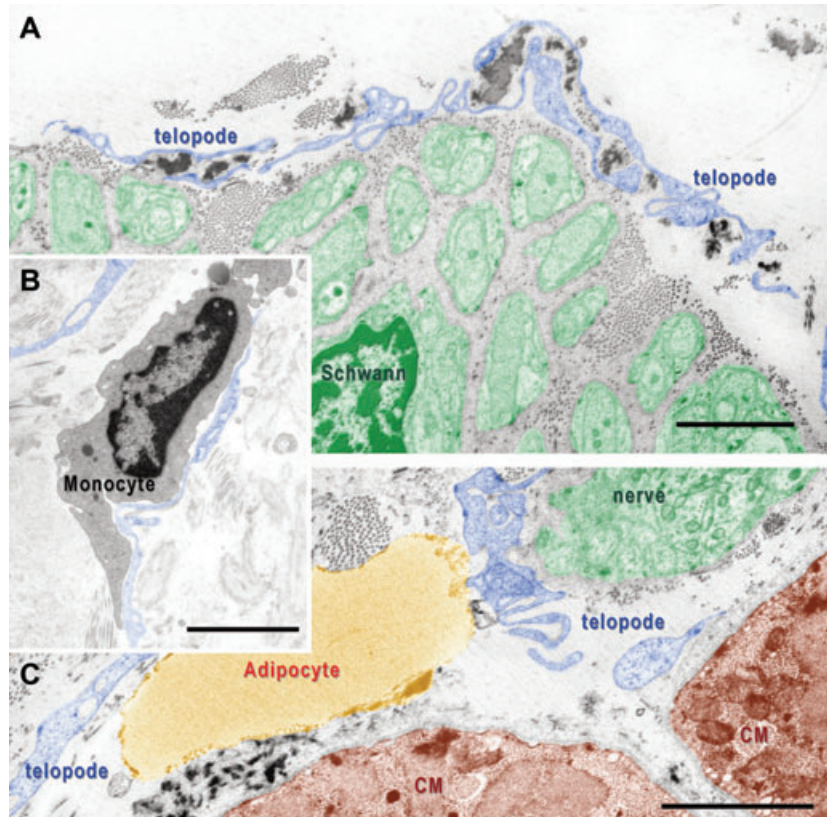


Fig. 6 Digitally coloured electron micrographs show telopodes (blue) and shed microvesicles (purple) in the extracellular matrix in subepicardium. (A) The telopode has a lacunar aspect and microvesicles seem to be released in the extracellular matrix or endocytosed into cytoplasmic pockets (arrows). (B) A telopode delivers few microvesicles (arrow) in the vicinity of a nerve fibre (green). Few caveolae (asterisks) are visible on plasma membrane of the telopode. (C) A multivesicular body (arrow) emerges from a telopode and discards its microvesicles (about 100 nm diameter) for another telopode. Arrowhead indicates a small attachment plaque connecting the telopode to extracellular matrix. Scale bar ~0.5µm.

Fig. 7 Electron micrographs of human subepicardium show that telopodes organized in a labyrinthine system connect nerves (green coloured in **A** and **C**), monocytes (**B**) and adipocytes (light brown, **C**). Cardiomyocytes (CM) are coloured in brown. Scale bar $-2 \mu\text{m}$.



think that telocytes may nurse the endogenous/ exogenous stem cells for activation and commitment, but also they act as supporting cells for progenitor cells migration towards injured myocardium [30].

Conflict of interest

The authors confirm that there are no conflicts of interest.

References

1. Zhou B, Pu WT. More than a cover: epicardium as a novel source of cardiac progenitor cells. *Regen Med.* 2008; 3: 633–5.
2. Manner J. Does the subepicardial mesenchyme contribute myocardioblasts to the myocardium of the chick embryo heart? A quail-chick chimera study tracing the fate of the epicardial primordium. *Anat Rec.* 1999; 255: 212–26.
3. Lie-Venema H, van den Akker NM, Bax NA, *et al.* Origin, fate, and function of epicardium-derived cells (EPDCs) in normal and abnormal cardiac development. *ScientificWorldJournal.* 2007; 7: 1777–98.
4. Zhang F, Pasumarthi KB. Ultrastructural and immunohistochemical characterization of undifferentiated myocardial cells in the developing mouse heart. *J Cell Mol Med.* 2007; 11: 552–60.
5. Zhou B, Ma Q, Rajagopal S, Wu SM, *et al.* Epicardial progenitors contribute to the cardiomyocyte lineage in the developing heart. *Nature.* 2008; 454: 109–13.
6. Perino MG, Yamanaka S, Li J, *et al.* Cardiomyogenic stem and progenitor cell plasticity and the dissection of cardiopoiesis. *J Mol Cell Cardiol.* 2008; 45: 475–94.
7. Habib M, Caspi O, Gepstein L. Human embryonic stem cells for cardiomyogenesis. *J Mol Cell Cardiol.* 2008; 45: 462–74.
8. Liang FS, Crabtree GR. Developmental biology: the early heart remodeled. *Nature.* 2009; 459: 654–5.
9. Sucov HM, Gu Y, Thomas S, *et al.* Epicardial control of myocardial proliferation and morphogenesis. *Pediatr Cardiol.* 2009; doi: 10.1007/s00246-009-9391-8.
10. Gittenberger-de Groot AC, Winter EM, Poelmann RE. Epicardium derived cells (EPDCs) in development, cardiac disease and repair of ischemia. *J Cell Mol Med.* 2010; 14 : 1056–60.
11. Carmona R, Guadix JA, Cano E, *et al.* The embryonic epicardium: an essential element of cardiac development. *J Cell Mol Med.* 2010; doi: 10.1111/j.1582-4934.2010.01088.x.
12. Fausone-Pellegrini MS, Bani D. Relationships between telocytes and cardiomyocytes during pre- and post-natal life. *J Cell Mol Med.* 2010; 14: 1061–3.
13. Beltrami AP, Barlucchi L, Torella D, *et al.* Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell.* 2003; 114: 763–76.

14. **Oh H, Bradfute SB, Gallardo TD, et al.** Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. *Proc Natl Acad Sci USA*. 2003; 100: 12313–8.
15. **Müller P, Beltrami AP, Cesselli D, et al.** Myocardial regeneration by endogenous adult progenitor cells. *J Mol Cell Cardiol*. 2005; 39: 377–87.
16. **Rubart R, Field LJ.** Cardiac regeneration: repopulating the heart. *Annu Rev Physiol*. 2006; 68: 29–49.
17. **Torella D, Ellison GM, Mèndez-Ferrer S, et al.** Resident human cardiac stem cells: role in cardiac cellular homeostasis and potential for myocardial regeneration. *Nat Clin Pract Cardiovasc Med*. 2006; 3: S8–13.
18. **Formigli L, Perna AM, Meacci E, et al.** Paracrine effects of transplanted myoblasts and relaxin on post-infarction heart remodeling. *J Cell Mol Med*. 2007; 11: 1087–110.
19. **Leri A, Hosoda T, Rota M, et al.** Myocardial regeneration by exogenous and endogenous progenitor cells. *Drug Discov Today Dis Mech*. 2007; 4: 197–203.
20. **Smart N, Risebro CA, Melville AA, et al.** Thymosin beta4 induces adult epicardial progenitor mobilization and neovascularization. *Nature*. 2007; 445: 177–82.
21. **van Tuyn J, Atsma DE, Winter EM, et al.** Epicardial cells of human adults can undergo an epithelial-to-mesenchymal transition and obtain characteristics of smooth muscle cells *in vitro*. *Stem Cells*. 2007; 25: 271–8.
22. **Winter EM, Grauss RW, Hogers B, et al.** Preservation of left ventricular function and attenuation of remodeling after transplantation of human epicardium-derived cells into the infarcted mouse. *Circulation*. 2007; 116: 917–27.
23. **Winter EM, Gittenberger-de Groot AC.** Epicardium-derived cells in cardiogenesis and cardiac regeneration. *Cell Mol Life Sci*. 2007; 64: 692–703.
24. **Nesselmann C, Ma N, Bieback K, et al.** Mesenchymal stem cells and cardiac repair. *J Cell Mol Med*. 2008; 12: 1795–810.
25. **Kajstura J, Urbanek K, Rota M, et al.** Cardiac stem cells and myocardial disease. *J Mol Cell Cardiol*. 2008; 45: 505–13.
26. **Riley PR.** The adult epicardium: realizing the potential for neovascular therapy. *Arterioscler Thromb Vasc Biol*. 2008; 28: 803–4.
27. **Wojakowski W, Kucia M, KaŹmierski M, et al.** Circulating progenitor cells in stable coronary heart disease and acute coronary syndromes: relevant reparatory mechanism? *Heart*. 2008; 94: 27–33.
28. **Clavel C, Verfaillie CM.** Bone-marrow-derived cells and heart repair. *Curr Opin Organ Transplant*. 2008; 13: 36–43.
29. **Hare JM, Chapparro SV.** Cardiac regeneration and stem cell therapy. *Curr Opin Organ Transplant*. 2008; 13: 536–42.
30. **Popescu LM, Gherghiceanu M, Manole CG, et al.** Cardiac renewing: interstitial Cajal-like cells nurse cardiomyocyte progenitors in epicardial stem cell niches. *J Cell Mol Med*. 2009; 13: 866–86.
31. **Gherghiceanu M, Popescu LM.** Human epicardium: ultrastructural ancestry of mesothelium and mesenchymal cells. *J Cell Mol Med*. 2009; 13: 2949–51.
32. **Noort WA, Sluijter JP, Goumans MJ, et al.** Stem cells from in- or outside of the heart: isolation, characterization, and potential for myocardial tissue regeneration. *Pediatr Cardiol*. 2009; 30: 699–709.
33. **Gherghiceanu M, Popescu LM.** Cardiomyocyte precursors and telocytes in epicardial stem cell niche: electron microscope images. *J Cell Mol Med*. 2010; 14: 871–7.
34. **Popescu LM, Fausone-Pellegrini MS.** TELOCYTES – a case of serendipity: the winding way from Interstitial Cells of Cajal (ICC), via interstitial Cajal-like cells (ICLC) to TELOCYTES. *J Cell Mol Med*. 2010; 14: 729–40.
35. **Suciu L, Popescu LM, Gherghiceanu M, et al.** Telocytes in human term placenta: morphology and phenotype. *Cell Tissue Organs*. 2010; doi: 10.1159/000319467.
36. **Hinescu ME, Popescu LM.** Interstitial Cajal-like cells (ICLC) in human atrial myocardium. *J Cell Mol Med*. 2005; 9: 972–5.
37. **Popescu LM, Gherghiceanu M, Hinescu ME, et al.** Insights into the interstitium of ventricular myocardium: interstitial Cajal-like cells (ICLC). *J Cell Mol Med*. 2006; 10: 429–58.
38. **Hinescu ME, Gherghiceanu M, Mandache E, et al.** Interstitial Cajal-like cells (ICLC) in atrial myocardium: ultrastructural and immunohistochemical characterization. *J Cell Mol Med*. 2006; 10: 243–57.
39. **Mandache E, Popescu LM, Gherghiceanu M.** Myocardial interstitial Cajal-like cells (ICLC) and their nanostructural relationships with intercalated discs: shed vesicles as intermediates. *J Cell Mol Med*. 2007; 11: 1175–84.
40. **Kostin S, Popescu LM.** A distinct type of cell in myocardium: interstitial Cajal-like cells (ICLC). *J Cell Mol Med*. 2009; 13: 295–308.
41. **Suciu L, Popescu LM, Regalia T, et al.** Epicardium: interstitial Cajal-like cells (ICLC) highlighted by immunofluorescence. *J Cell Mol Med*. 2009; 13: 771–7.
42. **Kostin S.** Myocardial telocytes: a specific new cellular entity. *J Cell Mol Med*. 2010; doi: 10.1111/j.1582-4934.2010.01111.x.
43. **Pucovský V.** Interstitial cells of blood vessels. *ScientificWorldJournal*. 2010; 10: 1152–68.
44. **Limana F, Bertolami C, Mangoni A, et al.** Myocardial infarction induces embryonic reprogramming of epicardial c-kit(+) cells: role of the pericardial fluid. *J Mol Cell Cardiol*. 2010; 48: 609–18.
45. **Limana F, Zacheo A, Mocini D, et al.** Identification of myocardial and vascular precursor cells in human and mouse epicardium. *Circ Res*. 2007; 101: 1255–65.
46. **Castaldo C, Di Meglio F, Nurzynska D, et al.** CD117-positive cells in adult human heart are localized in the subepicardium, and their activation is associated with laminin-1 and alpha6 integrin expression. *Stem Cells*. 2008; 26: 1723–31.
47. **Zaglia T, Dedja A, Candiotti C, et al.** Cardiac interstitial cells express GATA4 and control dedifferentiation and cell cycle re-entry of adult cardiomyocytes. *J Mol Cell Cardiol*. 2009; 46: 653–62.
48. **Tallini YN, Greene KS, Craven M, et al.** c-kit expression identifies cardiovascular precursors in the neonatal heart. *Proc Natl Acad Sci USA*. 2009; 106: 1808–13.
49. **Popescu LM, Gherghiceanu M, Cretoiu D, et al.** The connective connection: interstitial cells of Cajal (ICC) and ICC-like cells establish synapses with immunoreactive cells. Electron microscope study *in situ*. *J Cell Mol Med*. 2005; 9: 714–30.
50. **Gherghiceanu M, Hinescu ME, Andrei F, et al.** Interstitial Cajal-like cells (ICLC) in myocardial sleeves of human pulmonary veins. *J Cell Mol Med*. 2008; 12: 1777–81.
51. **Fausone Pellegrini MS, Thuneberg L.** Guide to the identification of interstitial cells of Cajal. *Microsc Res Tech*. 1999 47: 248–66.
52. **Ciontea SM, Radu E, Regalia T, et al.** c-kit immunopositive interstitial cells (Cajal-type) in human myometrium. *J Cell Mol Med*. 2005; 9: 407–20.
53. **Hinescu ME, Popescu LM, Gherghiceanu M et al.** Interstitial Cajal-like cells in rat mesentery: an ultrastructural and immunohistochemical approach. *J Cell Mol Med*. 2008; 12: 260–70.
54. **Popescu LM, Ciontea SM, Cretoiu D, et al.** Novel type of interstitial cell (Cajal-like) in human fallopian tube. *J Cell Mol Med*. 2005; 9: 479–523.

55. **Popescu LM, Ciontea SM, Cretoiu D.** Interstitial Cajal-like cells in human uterus and fallopian tube. *Ann NY Acad Sci.* 2007; 1101: 139–65.
56. **Hinescu ME, Ardeleanu C, Gherghiceanu M, et al.** Interstitial Cajal-like cells in human gallbladder. *J Mol Histol.* 2007; 38: 275–84.
57. **Quesenberry PJ, Aliotta JM.** The paradoxical dynamism of marrow stem cells: considerations of stem cells, niches, and microvesicles. *Stem Cell Rev.* 2008; 4: 137–47.
58. **Yuan A, Farber EL, Rapoport AL, et al.** Transfer of microRNAs by embryonic stem cell microvesicles. *PLoS ONE.* 2009; 4: e4722.
59. **Gnecchi M, Zhang Z, Ni A, et al.** Paracrine mechanisms in adult stem cell signaling and therapy. *Circ Res.* 2008; 103: 1204–19.
60. **Milei J, Fraga CG, Grana DR, et al.** Ultrastructural evidence of increased tolerance of hibernating myocardium to cardioplegic ischemia-reperfusion injury. *J Am Coll Cardiol.* 2004; 43: 2329–36.
61. **Lachman N, Vanker EA, Christensen KN, et al.** Pericardiectomy: a functional anatomical perspective for the choice of left anterolateral thoracotomy. *J Card Surg.* 2009; 24: 411–3.
62. **Di Meglio F, Castaldo C, Nurzynska D, et al.** Epicardial cells are missing from the surface of hearts with ischemic cardiomyopathy: a useful clue about the self-renewal potential of the adult human heart? *Int J Cardiol.* 2009; doi: 10.1016/j.ijcard.2008.12.137.
63. **Caamano S, Shiori A, Srauss Sh, et al.** Does sodium dodecyl sulfate wash out of detergent-treated bovine pericardium at cytotoxic concentrations? *J Hart Valve Dis.* 2009 18: 101–5.
64. **Zhang H, Chen H, Wang W, et al.** Cell survival and redistribution after transplantation into damaged myocardium. *J Cell Mol Med.* 2010; 14: 1078–82.