Telocytes as supporting cells for myocardial tissue organization in developing and adult heart

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Abstract

Recent evidence indicates that the adult heart contains sub-epicardial cardiogenic niches where cardiac stem cells and stromal support-ing cells reside together. Such stromal cells include a special population, previously identified as *interstitial Cajal-like cells* and recently termed *telocytes* because of their long, slender processes (*telopodes*) embracing the myocardial precursors. Specific stromal cells, pre-sumptively originated from the epicardium, have been postulated to populate the developing heart where they are thought to play a role in its morphogenesis. This study is designed to investigate the occurrence of telocytes in the developing heart and provide clues to bet-ter understand their role as supporting cells involved in the architectural organization of the myocardium during heart development. Our results showed that stromal cells with the immunophenotypical (vimentin, CD34) and ultrastructural features of telocytes were present in the mouse heart since early embryonic to adult life, as well as in primary cultures of neonatal mouse cardiac cells. These cells formed an extended network of telopodes which closely embraced the growing cardiomyocytes and appeared to contribute to the aggregation of cardiomyocyte clusters *in vitro*. In conclusion, the present findings strongly suggest that, during heart development, stromal cells identifiable as telocytes could play a nursing and guiding role for myocardial precursors to form the correct three-dimensional tissue pattern and contribute to compaction of the embryonic myocardial trabeculae. It is tempting to speculate that telocytes could be a novel, possible target for therapeutic strategies aimed at potentiating cardiac repair and regeneration after ischemic injury.

Keywords: myocardial development ● cardiomyocytes ● mouse ● interstitial Cajal-like cells ● cardiac stromal cells ● telocytes

Introduction

There is firm evidence that, during the development of tissues and organs, form is imprinted in the stromal compartment, thus lead-ing to the notion that the stroma can be considered not only as a mere packaging entity, supporting and protecting parenchymal cells, but also as a key regulator of tissue homeostasis, being involved in cell proliferation, survival, differentiation, metabolism and morphogenetic movements [1, 2]. In this line, peculiar stromal cells have been identified in the developing organs during pre-natal life and described as having numerous, long, thin cyto-plasmic processes – or *filopodes* – which can be extended and retracted to form a sort of scaffold for migration of parenchymal precursors towards their appropriate locations to form a complex tissue-specific architecture [3]. Such filopodes may correspond to the actin-containing cell extensions, called *cytonemes*, described in developing *Drosophila* and supposed to be responsible for some forms of long-range cell–cell communication aimed at coor-dination of morphogenesis [4]. Of note, stromal cells bearing long cellular extensions have been also described in most mature organs, although they have been long neglected and simplistically labelled as *fibroblasts*. However, this view is being rapidly chang-ing due to the recent observations by Popescu and coworkers who found stellate-shaped stromal cells occurring in several adult tis-sues and organs, where they may represent a distinct cell type from the classical fibroblasts [5]. Based on their morphological similarity with interstitial Cajal cells, the smooth muscle pace-maker cells of the gastrointestinal tract, these special stromal cells have been initially termed *interstitial Cajal-like cells*, although this denomination has been soon felt inappropriate. Recently, for these cells, the specific, descriptive term *telocytes* (*telos*, *i.e.* provided with long-distance cell projections) has been chosen, and their processes have been termed *telopodes* [6]. Indeed, their peculiar

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ultrastructural phenotype is currently considered as the only reliable hallmark for these cells, which do not possess a unique antigenic profile [5–7].

Interestingly, telocytes have been recently found in the human myocardium [5, 8] and, later on, identified in developing and mature hearts into specific microenvironmental sites [9], the epicardial cardiogenic niches located at the areas of coronary artery branching [10], intermingled with cardiac stem cells [5, 10]. The close interaction between these two cardiac cell types in the cardiogenic niches [10] has generated the interesting hypothesis that telocytes may be one of the cells involved in the cardiac morphogenesis [11].

It has been reported that the epicardium plays a pivotal role in heart morphogenesis, as it is a source of mitogenic factors promoting cardiomyocyte proliferation and of instructive signals that direct myocardial tissue organization [12]. Moreover, a subset of epicardial cells, the epicardium-derived cells (EPDCs), was seen to undergo epithelial–mesenchymal transition and migrate into the originally acellular subepicardial space and subsequently into the myocardium, where they differentiate into various supporting cell types to the myocardium, including for instance coronary smooth muscle cells, interstitial and perivascular fibroblasts [13, 14]. However, the exact nature and role of the cells present in the subepicardial space during the early embryonic life remains to be elucidated [15, 16]. We recently supposed that such cells could include the telocytes [11].

The aim of the present study was to provide further clues for the supporting role of telocytes in the architectural organization of the myocardium by studying their occurrence and relationships with cardiac muscle cells during cardiac organogenesis. To this purpose, we investigated morphologically the prenatal and postnatal mouse heart as well as primary co-culture of cardiac stromal and muscle cells isolated from newborn mice.

Materials and methods

In vivo studies

Samples of myocardium were obtained from embryonic (E14, E17, *e.g.* at 14- and 17-day pregnancies), newborn (P0, P6, *e.g.* at birth and at 6 days after delivery) and adult (2 months) CD1 mice (Harlan, Correzzana, Italy). The animals were housed at 22–24°C under a 12 hr light/12 hr dark cycle, with free access to standard laboratory chow (Harlan) and water. The experimental protocol was designed in compliance with the recommendations of the European Economic Community (86/609/CEE) for the care and use of laboratory animals and was approved by the Committee for Animal Care of the University of Florence (Italy). The embryos were collected from the uterine horns of females at 14 and 17 days of pregnancy, killed by decapitation and fixed for light microscopic immunohistochemistry and transmission electron microscopy (TEM), as detailed below. Similarly, the hearts from newborn and adult mice were taken at surgery, immediately dissected to isolate the ventricular area near to the atrioventricular sulcus and fixed for light and electron microscopic examination. Tissue sections of the above specimens were stained with haematoxylin and eosin for routine light microscopy examination.

In vitro studies

Hearts from 0- to 1-day-old newborn mice were used to obtain primary cultures of ventricular cardiac muscle and stromal cells, according to the previously described method [17]. In fact, by omitting a pre-plating step on collagen-coated culture plates, this isolation procedure yields a mixed population composed mainly of immature cardiomyocytes and stromal cells. These cells were then seeded and allowed to grow for 48 hrs on glass cover slips for light microscopic studies, or in cell culture inserts bottomed with collagen-coated cellulose membranes (Millicell HA, Millipore, Billerica, MA, USA) for electron microscopic studies The cultures were maintained in DMEM added with 10% horse serum and 5% foetal calf serum. Time-dependent changes in the features of the cardiac cultures were analysed by time-lapse videomicroscopy, as described [9]. At the 48 hr end-point, the remaining cultures were fixed and examined by confocal laser scanning fluorescence microscopy or TEM.

Immunohistochemistry

Heart specimens were fixed in 3% paraformaldehyde in PBS, pH 7.4, embedded in paraffin and cut in 4-μm-thick sections. For heat-induced antigen retrieval, the sections were treated for 20 min. at 90–92°C in 10 mM Tris buffer and 1 mM ethylenediaminetetraacetic acid, pH 9.0. After quenching of endogenous peroxidase with 3% H_2O_2, the sections were pre-incubated in 1% bovine serum albumin in PBS for 30 min. and then immunostained for 30 min. at room temperature with the following antibodies: rabbit polyclonal anti-CD117/c-kit (Dako, Milan, Italy; 1:400), mouse monoclonal anti-CD34 (Dako; 1:25), goat polyclonal anti-vimentin (Sigma, Milan, Italy). To minimize background staining due to non-specific binding of the secondary antibodies to endogenous mouse immunoglobulins, the sections incubated with the mouse anti-CD34 antiserum were previously treated with a goat antimouse Ig antiserum (Sigma, 1:50, 30 min.). Immune reaction was revealed by appropriate biotinylated anti-rabbit, antimouse or anti-goat secondary antisera (Vector, Burlingame, CA, USA) incubated for 90 min. at room temperature, followed by 20 min. incubation in avidin/biotin complex (Vector), using diaminobenzidine as chromogen. Sections not incubated with the primary antibody or incubated with non-immune rabbit, mouse or goat serum, as appropriate, were used as negative controls. Nuclei were counterstained with haematoxylin and the sections observed and photographed with a Zeiss Axioscop light microscope (Zeiss, Oberkochen, Germany). In parallel experiments, paraformaldehyde-fixed cultures of neonatal cardiac cells were also immunostained with the noted antisera. Immune reaction was revealed using Alexa-conjugated goat antimouse or anti-rabbit IgG (1:100; Molecular Probes, Eugene, OR, USA). Cells were examined with a Leica TCS SP5 confocal laser scanning microscope (Leica Microsystem, Mannheim, Germany) equipped with a HeNe/Argon laser source for fluorescence images and differential interference contrast optics for transmission images. A series of optical sections (1024 × 1024 pixels) at intervals of 0.8 μm were obtained and superimposed to create a single composite image.

Transmission electron microscopy

Full-thickness heart specimens or cardiac cell cultures grown for 48 hrs over the cellulose membranes of cell culture inserts were fixed in 2%

Fig. 1 Immunohistochemistry. (**A–C**) c-kit immuno-labelling. **A**, E14 embryo: the lining epicardial cells and the immature cardiomyocytes are intensely c-kit$^+$. Intermingled with the latter cells there are several c-kit$^-$ stellate cells featuring telocytes. (**B**) E17 embryo: the lining epicardial cells are still c-kit$^+$ (upper part) and the putative telocytes are c-kit$^-$ and appear to border the cardiomyocyte columns. (**C**) P0 newborn: cardiomyocytes are c-kit$^+$. However, c-kit staining intensity decreases with age (compare **C** and **B**). (**D–F**) CD34 immunolabelling. (**D**) E14 embryo: none of the cells is CD34$^+$. **E**, E17 embryo: the lining epicardial cells and several subepicardial cells, some of which identifiable as endothelial cells, are CD34$^+$. (**F**) P6 newborn: most of the endothelial cells as well as a few interstitial cells, likely identifiable as telocytes, are CD34$^+$. (**G–K**) Vimentin (vim) immunolabelling. (**G**) E14 embryo: several subepicardial cells intermingled with the immature cardiomyocytes are vimentin$^+$. (**H**) P0 newborn: vimentin$^+$ cells border the cardiomyocyte trabeculae. (**K**) P6 newborn: numerous vimentin$^+$ cells can be seen: some of them border and others are intermingled with the cardiomyocyte columns. (**I**) Adult: a typical telocyte (*arrow*) bordering a cardiomyocyte is CD34$^+$. (**J**) Adult: two vimentin$^+$ telocytes adjacent to cardiomyocytes. (**L**) Adult: none of the cells is c-kit$^+$. Ep: epicardial cells. Scale bar: **A**, **C** = 20 μm; **B**, **F**, **G–K** = 40 μm; **D**, **E**, **J** = 15 μm; **I**, **L** = 10 μm.
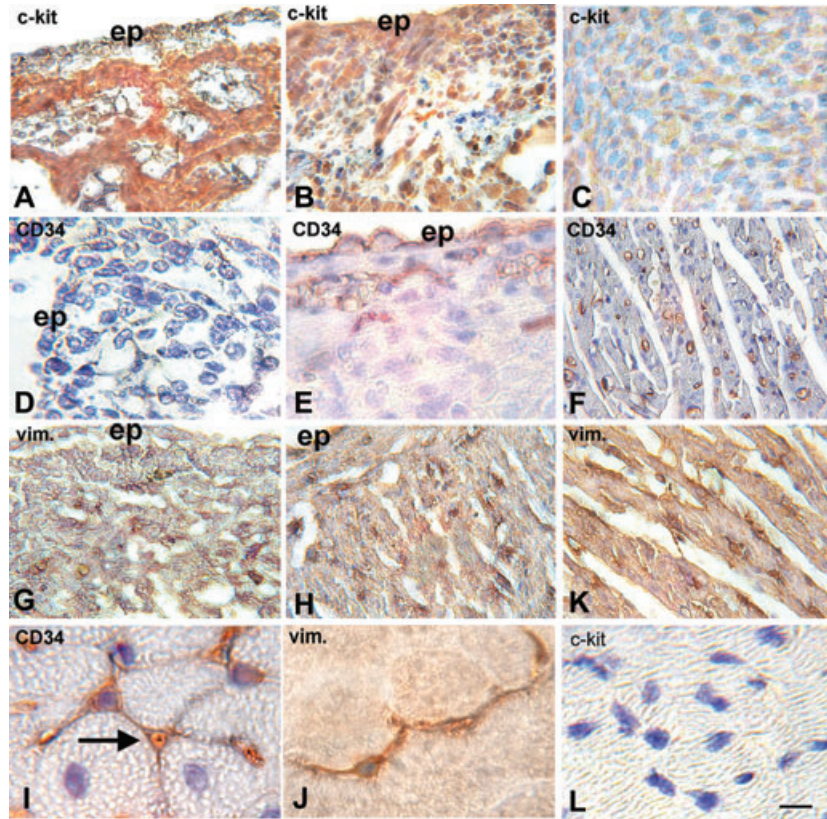
cacodylate-buffered glutaraldehyde, pH 7.4, for 6 hrs, post-fixed in 1% phosphate-buffered OsO$_4$, pH 7.4, for 2 hrs and embedded in epoxy resin. Semi-thin sections were obtained with a LKB NOVA ultra-microtome (LKB, Uppsala, Sweden), stained with toluidine blue in 0.1 M borate buffer, and observed under a light microscope. Ultra-thin sections of the areas of interest were stained with uranyl acetate and bismuth subnitrate and examined and photographed with a JEOL 1010 electron microscope (JEOL, Tokyo, Japan) and a Morgagni (FEI Company, Eindhoven, The Netherlands) electron microscope.

Results

In vivo studies on mouse heart specimens

Light microscopy

In the embryos, the heart had trabecular morphology. Both atria and ventricles had a wide lumen bordered by the endocardium and a continuous outer epicardial lining. Immature cardiomyocytes were present in the sub-epicardial region, arranged in columns protruding toward the heart lumen. These columns appeared longer and thicker in the ventricles than in the atria. By P0, this trabecular morphology of the heart was still appreciable, although the cardiomyocyte columns were larger and tended to com-paction, especially in the ventricle wall, as compared with the pre-natal stages. During late post-natal life, the heart acquired its typical mature morphological pattern. By immunohistochemistry, cells expressing the stemness marker c-kit could be found in all the specimens investigated, starting from the early embryonic to the post-natal life. In embryos and newborns, the epicardial and subepicardial layers appeared intensely c-kit$^+$ (Fig. 1A–C). In particular, the subepicardial layer was composed mainly by developing immature cardiomyocytes. C-kit immunostaining decreased along with cell differentiation (Fig. 1C), and appeared faint or almost undetectable in the adult cardiomyocytes (Fig. 1L). CD34$^+$ cells were not detected at E14, despite the presence of spindle- or stellate-shaped CD34$^-$ stromal cells, featuring putative telocytes (Fig. 1D). On the other hand, by E17 to post-natal life, CD34$^+$ cells could be found in the epicardial layer (Fig. 1E). Positive cells identifiable as endothelial cells were also observed in the subepicardial area at this age (Fig. 1E), as well as in the post-natal myocardium (Fig. 1F). At P0 and P6, cells featuring putative telocytes were typically located in the interstitium surrounding the cardiomyocytes, but many of them resulted negative for CD34 (Fig. 1F). In the adult hearts, CD34 immunostaining was mostly restricted to endothelial cells, with the exception of few, scattered stellate cells which could be identified as putative telocytes (Fig. 1I). Vimentin$^+$ cells were found in all the specimens examined in the subepicardial areas starting from E14 (Fig. 1G). At this developmental stage, they were
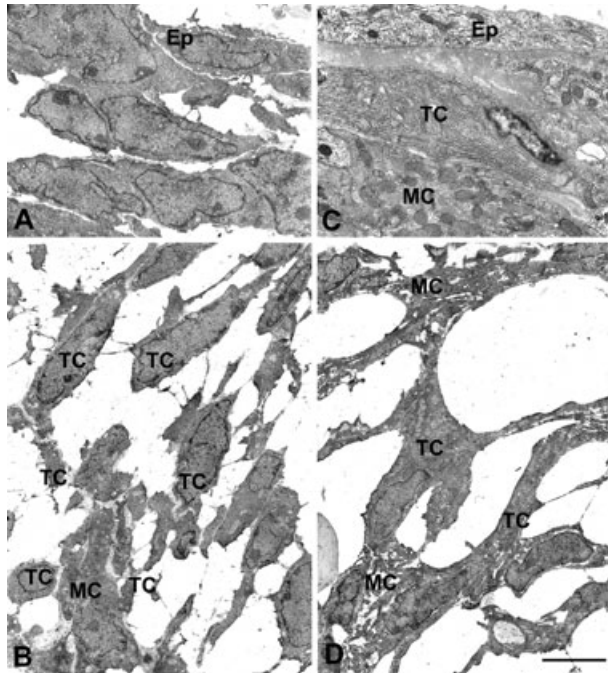
Fig. 2 TEM. E14 (**A**, **B**) and E17 (**C**, **D**) embryos. **A** and **C** show the epicardium and subepicardial area: presumptive telocytes have blast-like features with free ribosomes in the cytoplasm in the early embryos (**A**) and several rough endoplasmic reticulum cisternae in late embryos (**C**). In **B** and **D**, cells featuring telocytes are located in the wide spaces separating the columns of immature cardiomyocytes. By their long, thin processes, the telocytes contact and border the cardiomyocytes. Ep: epicardium; TC: telocytes; MC: cardiomyocytes. Scale bar: **A** = 1.8 μm; **B**, **D** = 1.6 μm; **C** = 1.2 μm.

Fig. 3 TEM. P0 newborn. (**A**) A stellate telocyte with undifferentiated features is seen in the interstitial space between cardiomyocytes, immersed in a loose extracellular matrix; its thin processes are contacting the neighbouring cardiomyocytes. (**B**) A telocyte showing a more differentiated phenotype, with several rough endoplasmic reticulum cisternae, is closely apposed to a cardiomyocyte (bottom) and a capillary (cap). The interstitial space is narrowed. (**C–E**) the telocytes establish numerous interactions (*arrows*) with the adjacent cardiomyocytes, in the form of focal plasma membrane contacts and intercellular bridges of flocculent, basal lamina-like material. In (**D** and **E**) the telocytes processes (*asterisks*) appear to mould the apposed cardiomyocytes to a bifurcated shape **TC**: telocytes. Scale bar: **A** = 1 μm; **B** = 1.2 μm; **C**, **D** = 0.6 μm; **E** = 0.5 μm.

few and scattered and tended to increase in number thereafter (Fig. 1H–K). In the post-natal and adult hearts, vimentin^{+} cells featuring telocytes were observed in the interstitium to border the myocardial trabeculae (Fig. 1G–K) and interposed between neighbouring cardiomyocytes (Fig. 1J).

Transmission electron microscopy

In the E14 and E17 embryos, numerous stellate cells featuring telocytes were observed in the subepicardial layer (Fig. 2A–D) intermingled with immature cardiomyocytes, which grew as elongated clusters protruding towards the cardiac lumen (Fig. 2B–D). These presumptive telocytes were provided with long processes emanating from an oval-shaped, small cell body which contained mainly free polyribosomes, and were surrounded by an electron-lucent amorphous matrix (Fig. 2B–D). In the P0 newborn heart, some putative telocytes retained a stellate morphology and were immersed in a loose extracellular matrix (Fig. 3A), while others showed a more differentiated phenotype, with several rough endoplasmic cisternae within the cell body, and two/three thin processes (Fig. 3B). These latter cells displayed the typical ultrastructural phenotype described previously for cardiac telocytes [5, 7, 9]. In the newborn heart, such telocytes were found closely
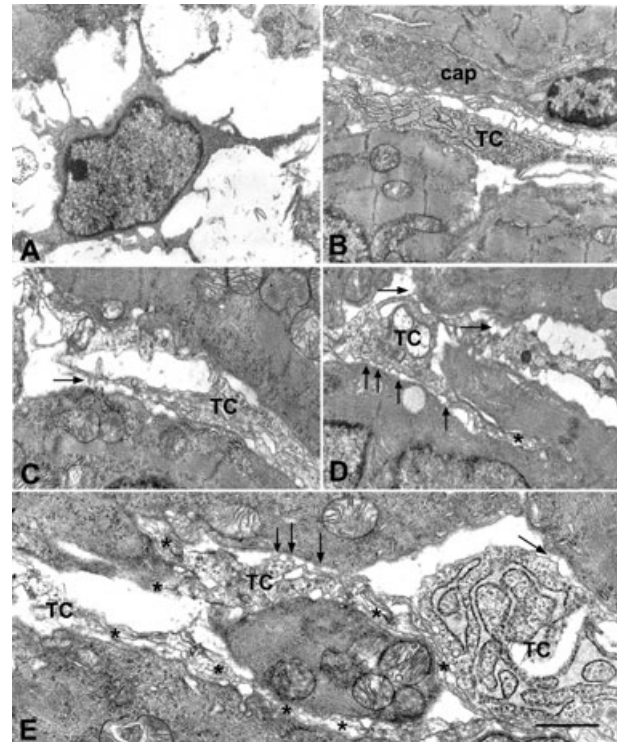
apposed to primitive endothelial cells, recognized by the presence of a basal lamina and pinocytosis vesicles (Fig. 3B). They were intermingled with coarse buds of growing cardiomyocytes and established numerous interactions with the latter cells, in the form of both focal plasma membrane contacts and intercellular bridges of flocculent, basal lamina-like material (Fig. 3C–E). In some instances, the cell processes appeared to mould the apposed cardiomyocytes to acquire a typical bifurcated shape (Fig. 3D and E). At P6, typical telocytes clearly bordered the cardiomyocyte columns (Fig. 4A and B). In the adult hearts, the telocytes had a similar ultrastructural appearance and localization as those found in the newborn hearts; at this stage, the most striking ultrastructural difference was the occurrence of fully differentiated, closely packed cardiomyocytes (Fig. 4A–C). Remarkably, multi-vesicular bodies apparently emanating from telopodes could be observed in the intercellular matrix (Fig. 4D), suggesting the possibility that
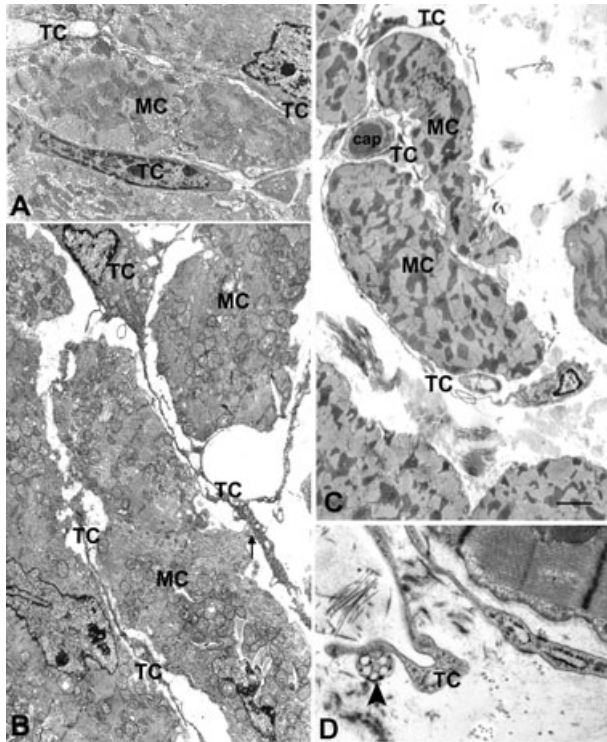
Fig. 4 TEM. P6 newborn (**A**, **B**) and adult (**C**, **D**). In (**A–C**) the telocytes with their bodies and long, thin telopodes embrace the adjacent cardiomyocytes. In (**D**) a membrane-bound multi-vesicular body featuring an exosome (*arrowhead*) is close to a telopode. TC: telocytes; MC: cardiomyocytes. Scale bar: **A**, **C** = 2.5 μm; **B** = 1.4 μm; **D** = 1 μm.

Fig. 5 Confocal immunofluorescent microscopy of newborn cardiac cell cultures. (**A**) Double labelling for c-kit (red) and vimentin (green): some vimentin$^+$ telocytes show long processes embracing the c-kit$^+$ cardiomyocytes. (**B**) Double labelling for c-kit (red) and CD34 (green): two CD34$^+$ cells with slender cytoplasmic processes are intermingled with the c-kit$^+$ cardiomyocytes. Scale bar: **A** and **B** = 10 μm.

telocytes could interact with the surrounding cardiomyocytes through the release of exosomes [10].

In vitro studies on newborn mouse cardiac cell cultures

Confocal microscopy

To test the hypothesis of a morpho-functional interaction between telocytes and developing cardiomyocytes, experiments were performed on primary cultures of neonatal cardiac cells. In keeping with our previous findings [10], the isolated cardiomyocytes grew as beating clusters and retained stemness features, such as the ability to proliferate and the surface expression of c-kit (Fig. 5A and B). Cells identified as telocytes, based on the presence of multiple telopodes and positive immunostaining for vimentin, formed an extended network surrounding and bridging the clusters of c-kit$^+$ immature cardiomyocytes (Fig. 5A). Scanty CD34$^+$ cells provided with thin processes extending between adjacent cardiomyocytes, possibly representing telocytes in a more advanced stage of differentiation, could also be observed within the clusters (Fig. 5B).
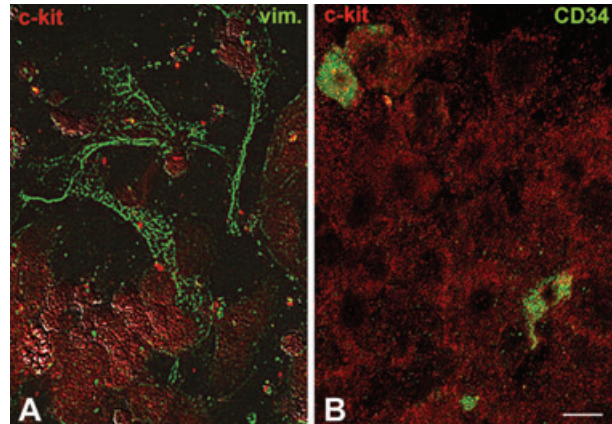
Transmission electron microscopy

Typical telocytes, substantially similar to those observed in the newborn and adult hearts, were commonly found within and around the cardiomyocyte clusters (Fig. 6A–D). In the clusters, telocytes contributed to the formation of a complex organotypic structure made up of alternate layers of these cells and differentiating cardiomyocytes (Fig. 6A–C). As observed in the developing hearts (Fig. 4B), these telocytes formed a complex three-dimensional telopode network embracing the growing cardiomyocytes (Fig. 6A and B). These latter cells showed immature features, *i.e.* few myofibrillae and abundant electron-lucent glycogenic fields (Fig. 6A–C), thus being similar to those found in the early embryonic hearts. Multi-vesicular bodies (Fig. 6A) and discrete sites of close plasma membrane apposition, gap junctions and patches of moderately electron-dense material between the two types of cells were often observed (Fig. 6D).

Time-lapse videomicroscopy

This dynamic approach allowed us to clarify the behaviour of telocytes during *in vitro* myocardial development. In fact, presumptive telocytes were commonly found among the cardiomyocyte clusters, often establishing cell–cell contacts with the peripheral cardiomyocytes. Notably, in a short time (2–3 hrs), these cells seemed to mediate the compaction of adjacent cardiomyocyte clusters, providing a guide and, possibly, the traction force for the gathering of cardiomyocytes into larger aggregates (Fig. 7A–E and Movie S1).

Discussion

The findings reported in this study indicate that a peculiar stromal cell type, the telocyte, is present in the mouse heart from embryonic
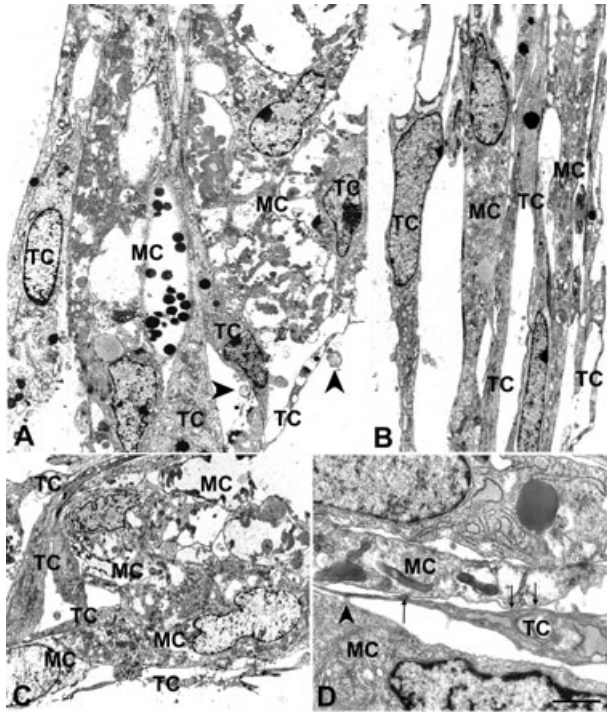
Fig. 6 TEM. Primary cultures of P0 newborn cardiac muscle and stromal cells. In (**A–C**) alternating layers of telocytes and immature cardiomyocytes are seen within the cardiac cell clusters (**A**, **C**) and at the clusters' periphery (**B**). A few myofibrillae and abundant electron-lucent glycogenic fields characterize the immature cardiomyocytes. In (**A**) multi-vesicular bodies (*arrows*) could be seen near telocytes' plasma membrane. (**D**) Sites of close plasma membrane apposition (*arrows*) and patches of moderately electron-dense material (*arrowhead*) between a telocyte and a cardiomyocyte. TC: telocyte; MC: cardiomyocyte. Scale bar: **A** = 2 μm; **B** = 3.3 μm; **C** = 4 μm; **D** = 0.8 μm.

Fig. 7 Time lapse phase-contrast videomicroscopy. Primary cultures of P0 newborn cardiac muscle and stromal cells. (**A–E**) images of the same microscopical field taken at 30 min. intervals: *arrowheads* point at some cells featuring telocytes that appear to mediate the compaction (*asterisks*) of the neighbouring cardiomyocyte clusters. Scale bar: 30 μm.

to adult life and appears to be involved in myocardial compaction. They also suggest that telocytes may play a role during myocardial development, possibly consisting in nursing and guiding cardiac muscle precursor cells to form the correct three-dimensional myocardial architecture. Indeed, by an integrated morphological approach, we confirm that telocytes are mainly recognizable based on their typical ultrastructural features and close relationships with the adjacent cardiomyocytes, as previously described by Popescu and coworkers [5–7, 10], and express a stromal cell-like immunophenotype, characterized by a widespread expression of vimentin and a discrete expression of CD34, the latter shared with endothelial cells. Since the embryonic developmental stage, telocytes were found to establish complex morpho-functional interactions with myocardial precursor cells. In particular, we showed that telocytes accompanied the proliferating cardiomyocyte buds moving from the epicardium to the ventricular lumen and, in the later stages, they bordered the myocardial trabeculae all along their length, suggesting a role for these cells in the creation of a proper milieu for the recruitment of cardiac precursors. As
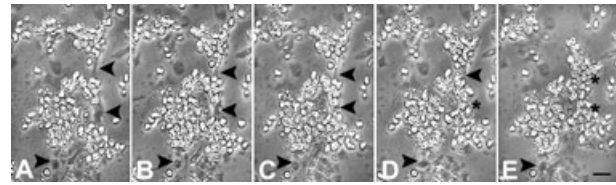
revealed by the ultrastructural analysis, telocytes also established close contacts with the cardiomyocytes, in the form of focal adhesions of the plasma membrane and intercellular bridges of basal lamina-like material, similarly to those observed in the cardiogenic niches of the adult heart [10]. Additional ways for the delivery of signalling molecules from telocytes to the surrounding cardiomyocytes could be operating through the release of multi-vesicular bodies featuring exosomes, similar to those described previously to have an important role in cardiac physiology and pathology [18–21]. These two ways of heterocellular communication (exosomes and structural contact) have been observed both *in vivo* and *in vitro* (Figs. 4 and 6).

Notably, by time-lapse videomicroscopy of cardiac cell cultures, the cells we identified as telocytes were seen to form a sort of three-dimensional scaffold which surrounded the cardiomyocyte clusters, to enhance and guide their compaction, thus highlighting the importance of stromal-derived signals and stromal–parenchymal interactions to induce maturation of cardiac cells till the formation of a correct myocardial-like structure [22, 23]. The present findings, obtained by morphological analysis combined with an *in vitro* dynamic study, taken together contribute to a better refinement of the concept that the stroma is a multi-functional compartment, capable of performing not only a mere passive mechanical support for the parenchyma, but also of giving an active contribution to tissue and organ shaping during development [1–3].

The embryonic development of the mammalian heart requires contributions from different cell types with specialized functions. It is generally accepted that the mesodermal precursors give rise to ventricular and atrial cardiomyocytes through two subsequent proliferations, or 'heart fields', whereas mesenchymal cells of the cardiogenic area, thought to be derived from the epicardium (EPDCs), give rise to the stromal components, including blood vessels, endocardium and valves [12–16, 24]. There is ample evidence that EPDCs are the source of the majority of the cells in the subepicardial space and, after migration into the developing myocardium, can give rise to vascular endothelial and smooth muscle cells and to adventitial and interstitial fibroblasts [15, 16]. This notion fits very well with our present data on the telocytes, which could well be a subpopulation of the EPDCs. In particular, the present study is consistent with the speculation that telocytes

might originate from c-kit/CD34⁻, vimentin⁺ precursors and acquire CD34 positivity with time. In the mature heart, we found that vimentin⁺ telocytes persist in the myocardium intermingled with cardiomyocytes. This is in agreement with previous observations reporting that telocytes reside in the myocardial interstitium [5, 6, 8], as well as in cardiogenic niches in close relationship with cardiac stem cells [10]. In these areas, at least some telocytes express the surface molecule CD34 [5, 8], which is also expressed by cells of the endothelial lineage [25]. Of note, during pre-natal heart development, we were unable to clearly distinguish between telocytes and endothelial cells by immunohistochemistry. Based on common mesenchymal origin, it cannot be ruled out that stromal precursor cells featuring telocytes could play the dual role of supporting cells for cardiomyogenesis and endothelial progenitors, capable to eventually differentiate into the microvascular framework of the mature myocardium.

Of note, EPDCs have also been shown to play a major role in three-dimensional organization of the heart and in formation of the compact myocardium. The current findings support the view that such roles can be attributed to the telocytes, thus supporting the hypothesis that these cells might actually be a subpopulation of EPDCs. Besides contributing to expand our knowledge on the mechanisms underlying myocardial development and allowing speculations on the putative role played by telocytes in this process, the present findings may be of relevance in the challenging field of cardiac regeneration, assuming that the same mechanisms of prenatal heart development may be re-activated in dormant myocardial precursors during the repair of the adult heart. Indeed, cardiac stem/progenitor cells are present in the adult heart [10, 26–28] but their scarce regenerative potential poses a major barrier to the functional restoration of the diseased myocardium, especially after massive tissue loss as occurs upon ischemic infarction [29]. Therefore, any strategies that could potentiate the attitude of these cells to proliferate, differentiate and establish functional connections with pre-existing cardiomyocytes, including modulation of the nursing properties of telocytes to influence the plasticity and developmental potential of cardiac progenitor cells, could offer new therapeutic targets for the management of the failing heart.

Conflict of interest

The authors confirm that there are no conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Movie S1 Time lapse videomicroscopy showing putative telocytes which mediate the compaction of adjacent cardiomyocyte clusters.

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