Annals of Anatomy 209 (2017) 37–44

Contents lists available at ScienceDirect

Annals of Anatomy

journal homepage: www.elsevier.com/locate/aanat

RESEARCH ARTICLE

Subsets of telocytes: Myocardial telocytes

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ARTICLE INFO

Article history:
Received 28 May 2016
Received in revised form
16 September 2016
Accepted 26 September 2016

Keywords:
Heart
Telopodes
Endothelial cells
Endothelial tip cells
CD34
Cardiac regeneration
Cardiac angiogenesis

ABSTRACT

Telocytes (TCs) are morphologically defined as small-sized cells with long, thin, moniliform processes called telopodes (Tps). Numerous papers imply that TCs are a distinctive cell type, and that transmission electron microscopy (TEM) is the gold standard tool for their identification. We aimed to reproduce previous studies on myocardial TCs to check their validity. For this purpose we performed an immunohistochemical study on human cardiac samples from six autopsied donor cadavers, using antibodies against CD10, CD31, CD34, CD146, Ki67, alpha-smooth muscle actin (α-SMA), Platelet-Derived Growth Factor Receptor—alpha (PDGFRα) and laminin. Additionally we performed a TEM study on cardiac samples from three human autopsied donor cadavers and five adult Sprague-Dawley rats.

We found endothelial cells (ECs), cords, and filopodia-projecting endothelial tip cells (ETCs) that expressed CD10, CD31, CD34, CD146, and PDGFR-α. Often, endothelial cells closely neighbored the sarcolemmal basal laminae. Endothelial progenitor cells, as well as nascent capillaries, were CD31+/CD34+. Proliferative endothelial cells expressed Ki67. In larger vessels we found pericytes that expressed CD146 and α-SMA; scarce α-SMA-expressing spindle-shaped cells lining cardiomyocytes were suggestive of a pericytic role in angiogenic sprout guidance.

The TEM study showed that endothelial tubes are almost exclusively found in the narrow myocardial interstitia. ECs that built them up appeared identical to the cells that previous TEM studies have suggested to be myocardial telocytes. A subset of stromal cells with TC-like phenotype and telopodes-like processes actually seem to configure blood vessels, and therefore belong to the endothelial lineage.

This study shows that data presented in previous studies on myocardial telocytes is not enough to allow the reproducibility of the results. At least a subset of cells considered to be TCs might belong to the endothelial lineage.

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1. Introduction

In 2010, Popescu and Faussone-Pellegrini reported, in an Editorial from the Journal of Cellular and Molecular Medicine, the identification of a new stromal cell type, the *telocyte* (TC) which was previously termed in studies of that group "*interstitial Cajal-like cell* (ICLC)" (Popescu and Faussone-Pellegrini, 2010). Since then numerous articles have been published about these small-sized stromal cells (Rusu et al., 2014, 2011, 2012a; Shi et al., 2016; Tao et al., 2015; Vannucchi et al., 2013).

TCs were finally defined by their morphological phenotype, as being "cells with telopodes" (Cretoiu and Popescu, 2014; Faussone Pellegrini and Popescu, 2011). Telopodes are long, slender, moniliform and eventually convoluted prolongations (Faussone Pellegrini and Popescu, 2011; Petre et al., 2015) of these TCs. Currently, although various attempts were made, a specific panel of markers was not assigned to TCs, these cells being accurately identified only by transmission electron microscopy (TEM) (Popescu and Faussone-Pellegrini, 2010). The gold standard for the identifi-

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http://dx.doi.org/10.1016/j.aanat.2016.09.006
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Table 1
Stem/progenitor markers used for the immunohistochemical study.

Markers	Specificity	Expression in telocytes
CD10	HSCs, MSCs (Petre et al., 2015)	± (Petre et al., 2015)
CD31	ECs, monocyte-derived ECs (Schatteman and Awad, 2004)	–
CD34	HSCs, EPCs, monocyte-derived mesenchymal progenitors	+
CD117/c-kit	HSCs, EPCs, monocyte-derived mesenchymal progenitors	±
CD146	MSCs	?
PDGFR-α	MSCs, HSCs (Fortini et al., 2011)	+ (Vannucchi et al., 2013)
α-SMA	Pericytes, MSC-like cells	+
Ki67	Proliferative cells	+ (Bojin et al., 2011)

MSC: mesenchymal stem cell; HSC: hematopoietic stem cell; EPC; endothelial progenitor cell; EC: endothelial cell; (?): not tested.

cation of TCs in TEM includes characteristics of the cell body and the telopodes (Popescu and Faussone-Pellegrini, 2010). The nucleus contains clustered peripheral heterochromatin while the small amount of cytoplasm of the cell is rich in mitochondria, has a small Golgi complex, rough and smooth endoplasmic reticulum and filaments, both intermediate and thin (Popescu and Faussone-Pellegrini, 2010). TCs display subplasmalemmal caveolae, and a basal lamina that may be either present or absent (Popescu and Faussone-Pellegrini, 2010). Telopode dilations were named podoms and are united by thin segments termed podomers (Cretoiu and Popescu, 2014; Rusu, 2014); podoms accommodate mitochondria, endoplasmic reticulum and display caveolae (Suciu et al., 2010).

In 2010, when TCs were defined as a new cell type, Kostin (2010) published a point of view, in which he implied that TEM is the only tool than can positively identify TCs; the focus of the study were myocardial TCs, which were identified next to cardiomyocytes, as presented in Supplemental Fig. S1 (reproduced with permission from John Wiley and Sons) (Kostin, 2010). The authors assumed a role of TCs in cardiac repair (Kostin, 2010). This has been supported by other studies suggesting a progenitor phenotype for TCs, which are reliably labeled by CD34 and vimentin (Diaz-Flores et al., 2015a,b, 2014). The functional role of TCs is purely hypothetical (Varga et al., 2016) and should be analyzed by further research: "*in the future, more functional studies on TCs in vivo will help understanding the mechanism by which TCs contribute to cardiac repair.*" (Tao et al., 2015).

The immunophenotype of the TCs has been intensely studied in the last years; different authors found that they express vimentin, CD34, Platelet-Derived Growth Factor Receptor—alpha (PDGFRα) or alpha-smooth muscle actin (α-SMA) (Vannucchi et al., 2013; Xiao et al., 2016; Zhou et al., 2015). c-Kit expression in TCs is rather heterogeneous. It should be kept in mind that TCs were initially considered to be interstitial Cajal-like cells, for which c-kit was a highly specific marker, in a similar fashion to the gastrointestinal interstitial Cajal cells.

Nevertheless, cardiac fibroblasts (FBs) are an accepted presence within cardiac interstitia. This leads us to recommend caution when distinguishing between FBs of TCs or of spindle-shaped progenitors. This is because hybrid morphologies which, depending on the cuts, could be erroneously diagnosed as TCs in TEM (Rusu et al., 2012b). We previously discussed this and consider that for a TC diagnosis in TEM the telopodial emergence, directly thin, from the cell body, could help distinguish telopodes of FBs thin processes (Rusu et al., 2012b).

The hypothesis of this study is that, at least the subset of cardiac TCs are in fact progenitor cells, most probably of the endothelial lineage. We, therefore, aimed to reproduce previous studies on myocardial TCs to check their validity, and to test the compliance with immunohistochemical criteria for cells belonging to the endothelial lineage. For the immunohistochemical study, we decided to use primary antibodies which specifically label distinct subclasses of stem/progenitor cells (Table 1).

2. Material and methods

For the immunohistochemical studies, adequately preserved human postmortem tissue was used, obtained during the autopsy of six cadavers (1:1 sex ratio) with ages varying between 28 and 82 years. Samples from three additional donor cadavers (two male, one female, of 34, 42 and, respectively, 46 years old) were used to document the ultrastructural study.

We also used five adult Sprague-Dawley rats weighing 350–400 g. The animals were kept in individual metabolic cages, under feeding and metabolism control, in standard lighting conditions. After preanaesthesia with ether, the animals were euthanized by intracardiac injection of a veterinary euthanasia drug (0.2 ml of T-61, INTERVET, Kirkland, Quebec, Canada). All procedures were approved by the Institutional Bioethics Committee.

2.1. Immunohistochemical study

The study was conducted according to the national laws regarding the use of cadaveric material for research purposes (including Law 104/2003 relating to the manipulation of human cadavers), and the general principles of the Declaration of Helsinki, Rio de Janeiro revision.

Tissue samples were adequately preserved in buffered formalin (8%), being further embedded in paraffin and prepared for immunohistochemistry. Paraffin blocks were processed with an automatic histoprocessor (Diapath, Martinengo, BG, Italy). Sections were cut manually at 3 μm and mounted on SuperFrost® electrostatic slides for immunohistochemistry (Thermo Scientific, Menzel-Gläser, Braunschweig, Germany). Histological evaluations used 3–4 μm thick sections stained with hematoxylin and eosin.

We used primary antibodies to CD10 (mouse monoclonal, clone 56C6, Biocare Medical, Concord, CA, USA, 1:20); CD117/c-kit (rabbit monoclonal, clone Y145, Biocare Medical, Concord, CA, USA, 1:100); CD146 (mouse monoclonal, clone N1238, Novocastra-Leica, Leica Biosystems Newcastle Ltd., Newcastle Upon Tyne, U.K., 1:50); CD31 (PECAM-1) (mouse monoclonal, clone BC2, Biocare Medical, Concord, CA, USA, 1:200); CD34 (clone QBEnd/10, Biocare Medical, Concord, CA, USA, 1:50); Ki67 (mouse monoclonal, clone MM1, Biocare Medical, Concord, CA, USA, 1:100); Platelet Derived Growth Factor Receptor-alpha (PDGFR-α, mouse monoclonal, clone C-9, Santa Cruz Biotechnology, Inc., Heidelberg, Germany, 1:100); α-smooth muscle actin (α-SMA, mouse monoclonal, clone 1A4, Dako, Glostrup, Denmark, 1:50); laminin (mouse polyclonal, BioGenex, Fremont, CA, USA, RTU).

Sections were deparaffinised, rehydrated and rinsed in PBS buffer solution at pH 7.4. Retrieval by incubation in specific buffer was completed as follows: (a) for CD34: EDTA, pH 9; (b) for the other antibodies: 0.01 M citrate retrieval solution, pH 6. The standard ABC technique used a DAB protocol (Rusu et al., 2009, 2011). Appropriate blocking of endogenous peroxidase was completed before immune labeling (Peroxidazed 1, Biocare Medical, Concord, CA, USA). Sections incubated with non-immune serum served

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