Telocytes in liver regeneration: possible roles

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Abstract

Telocytes (TCs) are a novel type of interstitial cells which are potentially involved in tissue regeneration and repair (www.telocytes.com). Previously, we documented the presence of TCs in liver. However, the possible roles of TCs in liver regeneration remain unknown. In this study, a murine model of partial hepatectomy (PH) was used to induce liver regeneration. The number of TCs detected by double labelling immunofluorescence methods (CD34/PDGFR-a, CD34/PDGFR-ß and CD34/Vimentin) was significantly increased when a high level of hepatic cell proliferation rate (almost doubled) as shown by 5-ethynyl-2'-deoxyuridine (EdU) immunostaining and Western Blot of Proliferating cell nuclear antigen (PCNA) was found at 48 and 72 hrs post-PH. Meanwhile, the number of CK-19 positive-hepatic stem cells peaked at 72hrs post-PH, co-ordinating with the same time-point, when the number of TCs was most significantly increased. Taken together, the results indicate a close relationship between TCs and the cells essentially involved in liver regeneration: hepatocytes and stem cells. It remains to be determined how TCs affect hepatocytes proliferation and/or hepatic stem cell differentiation in liver regeneration. Besides intercellular junctions, we may speculate a paracrine effect via ectovesicles.

Keywords: telocytes ● liver regeneration ● hepatocytes ● CK-19 positive-hepatic stem cells ● CD34 ● PDGFR-a, ß ● EdU ● PCNA

Introduction

Liver possesses a remarkable capacity to regenerate after toxic injury, virus infection, ischaemia and surgical resection [1]. Liver regeneration is typically mediated by the proliferation of remaining cells, with hepatocytes as the first to enter cell cycle and reach the peak of DNA synthesis, while non-parenchymal cells (Kupffer cells, biliary epithelial cells, hepatic stellate cells and endothelial cells, etc.) are later to proliferate [2]. Unfortunately, the proliferative capacity of hepatocytes is usually impaired upon severe or chronic liver injuries. In such circumstances, liver stem/progenitor cells are assumed to be activated and contribute to liver regeneration by differentiating to mature cells [3]. Considering that different types of hepatic cells participate in liver regeneration in an orchestrate manner, understanding how these cells work together as well as the regulatory mechanisms for their biological processes will help advance the basic knowledge on molecular and cellular aspects of liver regeneration.

Our group has previously documented the presence in mice liver of a distinct type of interstitial cell termed telocyte (TC) [4]. TCs were firstly identified by Popescu’s group [5–11], and then have been documented in the interstitial space of various organs and tissues in mammals [12–20]. TCs are characterized by a small cell body and extremely long prolongations named telopodes (Tps) with an alteration of thin segments (podomers) and dilated segments (podoms) [21–26]. Besides the established microRNA signatures [27] and gene profiles [28], the proteomic features [29] as well as chromosome 1 gene profile...
[30] of TCs have been identified. Increasing evidence indicated the potential roles of TCs in tissue regeneration and repair (heart, muscle and skin) by forming a complex network with neighbour cells and releasing shed vesicles and exosomes, thus regulating intercellular signalling that might be essentially involved in regeneration/repair [7, 8, 16, 17, 25, 31–34]. However, it remains to be determined the possible roles of TCs in the control of liver regeneration.

Thus, the aims of the present study were to investigate the roles of TCs in liver regeneration by using a murine model of partial hepatectomy (PH).

Materials and methods

Animals

Eight-week-old specific pathogen-free male C57BL/6 mice were purchased from SLAC Laboratory Animal Center, Shanghai (Shanghai, China). Mice were maintained in a temperature-controlled room on a 12 hrs light/dark cycle, with free access to water and standard chow. Mice were anaesthetized by 1% pentobarbital sodium intraperitoneal injection (50 mg/kg). Seventy Percent PH was performed on anaesthetized mice by removing the median and left lobes of the liver. At various time-points post-PH (0, 12, 24, 48, 72, 120 and 168 hrs), totally 40 mice were killed (five mice for every group). The mice of 0 hr (quiescent liver) group were used as baseline. Residual liver lobes and body weight were weighed at the time of killing, and the ratio of residual liver lobes weight to body weight was calculated to evaluate the regeneration of liver mass. Frozen liver sections were used for immunofluorescent staining and EdU (5-ethyl-2′-deoxyuridine) immunostaining. The rest of liver tissues were collected and stored at −80°C for Western blot analysis. This study was approved by the local ethical committees and all animal experiments were conducted under the established guidelines on the use and care of laboratory animals for biomedical research published by National Institutes of Health (No. 85-23, revised 1996).

EdU immunostaining

Mice were injected intraperitoneally with 50 mg/kg of EdU (C10314-3; Riobio, Guangzhou, China) 1 hr before killed. After washed with PBS for 15 min., frozen sections were fixed in paraformaldehyde for 30 min. After fixed, frozen sections were washed with PBS for three times, and then incubated with Cell-Light EdU Apollo® 488 In Vivo Imaging Kit (C10314-3; Riobio). After incubation with EdU kit, sections were washed with PBS for three times, then incubated with DAPI (4′,6-diamidino-2-phenylindole, 1:500 dilution; Life Technology). The same protocol was used in Rabbit monoclonal to PDGF Receptor-α (ab32570; Abcam) and rat monoclonal anti-CD34/b-actin (ab61219; Abcam, Cambridge, UK) and rat monoclonal anti-CD34/b-actin (ab8158; Abcam) primary antibodies. Both antibodies were diluted by 1:100 in 1× PBS with 0.25% Triton X-100. After that, sections were exposed for 1 hr to goat anti-rat labelled with FITC (sc-2011; Santa Cruz, Dallas, TX, USA) and goat anti-rabbit labelled with rhodamine (sc-362262; Santa Cruz) secondary antibodies diluted 1:200 in the same buffer. Finally, sections were stained with DAPI (ProLong® Gold; Life Technology). The same protocol was used in Rabbit monoclonal to PDGF Receptor-β (ab32570; Abcam) and rat monoclonal anti-CD34 (ab8158; Abcam) double labelling staining, and Rabbit monoclonal to Vimentin (ab92547, 1:100; Abcam) and rat monoclonal anti-CD34 (ab8158; Abcam) double labelling staining. The images were taken under an amplification of 400× with confocal laser scanning microscope (LSM 710; Carl Zeiss Microlaging GmbH). For detection of hepatic stem cells, immunofluorescent staining for CK-19 was conducted according to the same protocol as described above except that rabbit monoclonal anti-CK-19 primary antibody (ab52625, 1:50; Abcam) was used instead.

Western blot analysis

Liver tissues were lysed with RIPA lysis buffer (Beyotime Institute of Biotechnology, Haimen, China). Equal amounts of 30 μg of total protein were subjected to electrophoreses on 10% SDS-PAGE gels, transferred to PVDF membranes and incubated with anti-PCNA (Proliferating cell nuclear antigen, 10205-2-AP, 1:1000; Proteintech, Chicago, IL, USA) or anti-β-actin (M1210-2, 1:1000; HuAan, Hangzhou, China) primary antibodies. After incubated with the corresponding HRP-conjugated secondary antibodies, protein bands were visualized using enhanced chemiluminescence system (Pierce Biotechnology Inc., Rockford, IL, USA) with ChemiDoc XRS Plus luminescent image analyzer (Bio-Rad, Hercules, CA, USA). Densitometric analysis of protein bands was performed with Image Lab software (Bio-Rad). Loading volume of each sample was normalized by β-actin protein band density.

Immunofluorescent staining

For detection of TCs in liver, double immunofluorescent staining for CD34/PDGFR-α or CD34/PDGFR-β or CD34/Vimentin was used. Briefly, frozen sections (6 μm thick) were mounted on Super frost Plus slides (Shittai, China), and then fixed in paraformaldehyde for 15 min. After washed with PBS for three times, sections were pre-incubated in PBS supplemented with 10% goat serum for 1 hr, and then incubated overnight at 4°C with rabbit polyclonal anti-PDGF Receptor-α (ab61219; Abcam, Cambridge, UK) and rat monoclonal anti-CD34 (ab8158; Abcam) primary antibodies. Both antibodies were diluted by 1:100 in 1× PBS with 0.25% Triton X-100. After that, sections were exposed for 1 hr to goat anti-rat labelled with FITC (sc-2011; Santa Cruz, Dallas, TX, USA) and goat anti-rabbit labelled with rhodamine (sc-362262; Santa Cruz) secondary antibodies diluted 1:200 in the same buffer. Finally, sections were stained with DAPI (ProLong® Gold; Life Technology). The same protocol was used in Rabbit monoclonal to PDGF Receptor-α (ab32570; Abcam) and rat monoclonal anti-CD34 (ab8158; Abcam) double labelling staining, and Rabbit monoclonal to Vimentin (ab92547, 1:100; Abcam) and rat monoclonal anti-CD34 (ab8158; Abcam) double labelling staining. The images were taken under an amplification of 400× with confocal laser scanning microscope (LSM 710; Carl Zeiss Microlaging GmbH). For detection of hepatic stem cells, immunofluorescent staining for CK-19 was conducted according to the same protocol as described above except that rabbit monoclonal anti-CK-19 primary antibody (ab52625, 1:50; Abcam) was used instead.

Statistical analysis

All analyses were evaluated using SPSS 19.0. Data are expressed as mean ± SEM. For parametric data, statistical significance was determined with one-way ANOVA test followed by two-tailed Student’s t-test. For non-parametric data, statistical significance was determined with Mann–Whitney U-test. 95% confidence intervals were presented. P < 0.05 was considered statistically significant.

Results

As shown in Figure 1A, the ratio of residual liver lobes weight to body weight was gradually elevated within 168 hrs post-PH. EdU immunostaining was performed to further investigate the proliferative effect of liver regeneration post-PH. As shown in Figure 1B, the number of EdU-positive cells/mm² was significantly increased at 48 hrs.
Double-positive cells was observed at 48 hrs of CD34/PDGFR-ß double-positive cells was observed at 48 hrs of CD34/PDGFR-ß double-positive cells was observed at 48 hrs post-PH, co-ordinating with the time-point when the number of TCs was most significantly increased. These results indicate a close relationship between TCs and hepatocytes and/or stem cells essentially involved in liver regeneration.

Liver regeneration is a highly orchestrate response to the loss of effective liver mass after liver resection or toxic injury [1]. The proliferation of remaining hepatocytes and the differentiation of liver stem cells are considered two main mechanisms for new hepatocyte production [2, 3]. CK-19 is a typical marker for adult hepatic stem cells that are activated during liver injuries [3]. Our present study demonstrated a peak of cell proliferation 48–72 hrs post-PH in mice, accompanied by an elevated number of TCs in liver. This suggests that TCs may be probably involved in the proliferative capacity of hepatocytes during liver regeneration. TCs were found to be connected with other cells including stem cells, for example in heart [5], lungs [24], liver [4], skin [8], eye [11], etc. However, the roles of TC in the proliferative capacity of hepatocytes remain to be further clarified in liver regeneration.

In the present study, the increased number of TCs 72 hrs after PH was accompanied by a corresponding increase in hepatic stem cells. Previous studies documented the presence of TCs in heart, lung, liver, skeletal muscle, skin and eye in tandem with stem cells [4, 5, 8, 11, 24, 25]. TCs are responsible for releasing shed vesicles and exosomes, regulating the transcriptional activity and activation of stem cells, thus contributing to stem cell-mediated tissue regeneration/repair [7, 8, 17, 24, 25, 35]. Under certain severe or chronic injury conditions, the differentiation of hepatic stem cells to mature cells represents another mechanism of liver regeneration [3], whereas it remains to be determined how TCs exert the functional effects on hepatic stem cell-mediated liver regeneration.

Several limitations of the present study should be highlighted. TCs, as a new type of interstitial cells, are characterized by extremely long and thin prolongations called Tps extending from the cell body [5]. Although hepatic TCs are reside in Disse’s space like hepatic stellate cells, TCs have very long Tps as indicated in our previous work [4] and also have specific biomarkers (double-positive for CD34 and Vimentin) were conducted. The number of CD34/PDGFR-ß double-positive cells was significantly increased at 72 hrs (P = 0.012, 95% CI = (0.42, 2.57)) post-PH (Fig. 2), and significant increased number of CD34/PDGFR-ß double-positive cells was observed at 48 hrs (P = 0.006, 95% CI = (1.49, 6.12)) and 72 hrs (P = 0.001, 95% CI = (4.46, 11.53)) post-PH (Fig. 3), while the increase in CD34/Vimentin double-positive cells was observed at 48 hrs (P = 2.36 × 10^{-18}, 95% CI = (25.38, 35.63)), 72 hrs (P = 1.36 × 10^{-22}, 95% CI = (45.16, 54.84)), 96 hrs (P = 1.53 × 10^{-16}, 95% CI = (24.41, 34.09)) and 120 hrs (P = 1.87 × 10^{-9}, 95% CI = (9.91, 19.59); Fig. 4), corresponding to the proliferative peak time-point of liver regeneration post-PH.
Fig. 2 Detection for TCs by double labelling immunofluorescence methods (CD34/PDGFR-α). Detection for TCs by CD34/PDGFR-α double immunofluorescence labelling in liver post-PH. Confocal laser scanning microscopy: double labelling immunofluorescence shows CD34 (green) and PDGFR-α (red) double-positive cells (pointed with arrows). Nuclei were counterstained with DAPI (blue). Original magnification 400 x; scale bar = 20 μm. *P < 0.05.

Fig. 3 Detection for TCs by double labelling immunofluorescence methods (CD34/PDGFR-β). Detection for TCs by CD34/PDGFR-β double immunofluorescence labelling in liver post-PH. Confocal laser scanning microscopy: double labelling immunofluorescence shows CD34 (green) and PDGFR-β (red) double-positive cells (pointed with arrows). Nuclei were counterstained with DAPI (blue). Original magnification 400 x; scale bar = 20 μm. *P < 0.05.
[27–29], the comparisons of microRNA signature, gene profile and proteome between hepatic TCs and hepatic stellate cells are needed. In addition, liver regeneration following PH is mainly achieved by hepatocyte replication although a small yet significant proportion of newly formatted hepatocytes may generated from hepatic stem cells [37]. Interestingly, based on the results from our study, the peak

**Fig. 4** Detection for TCs by double labelling immunofluorescence methods (CD34/Vimentin). Detection for TCs by CD34/Vimentin double immunofluorescence labelling in liver post-PH. Confocal laser scanning microscopy: double labelling immunofluorescence shows CD34 (green) and Vimentin (red) double-positive cells (pointed with arrows). Nuclei were counterstained with DAPI (blue). Original magnification 400×; scale bar = 20 μm. *P < 0.05.

[Diagram with CD34 and Vimentin staining at different time points: 0 hr, 12 hr, 24 hr, 48 hr, 72 hr, 96 hr, 120 hr.]

**Fig. 5** Detection for hepatic stem cells by immunofluorescent staining for CK-19. Representative images of CK-19 (green) positive cells in liver (pointed with arrows). Nuclei were counterstained with DAPI (blue). Original magnification 400×; scale bar = 20 μm. *P < 0.05.

[Diagram with CK-19 staining at different time points: 0 hr, 12 hr, 24 hr, 48 hr, 72 hr, 96 hr, 120 hr.]
activity of cell replication was at 48 hrs, whereas the peak in TCs and stem cell activity was at 72 hrs (although there was considerable overlap), making it possible that TCs might have a more important role in regeneration mediated by hepatic stem cells compared to that by hepatocytes. Thus, it would be interesting to inhibit hepatocyte proliferation using 2-acetylaminofluorene before PH (2AAF/PH) to identify the role of TCs in liver regeneration mediated mainly by the activation of hepatic stem cells [37]. Moreover, a direct evidence of relationship between TCs and CK-19 positive cells is also required in the future.

In conclusion, our study indicates a potential role of TCs in liver regeneration, probably through their close relationship with hepatocytes and hepatic stem cells or a paracrine effect via ectovesicles. Three possibilities of the relationship between TCs and the proliferating cells in PH could be proposed: (i) the co-ordinating increase in TCs and proliferating cells in PH is just a coincidence; (ii) the increased TCs is just associated with the increase in proliferating cells in PH and (iii) the increased TCs is the cause for the increase in proliferating cells in PH. Considering that CD34/PDGFR-α, CD34/PDGFR-β and CD34/Vimentin positive TCs after PH increase with an accompanying increase in hepatocytes and hepatic stem cells, we speculate that TCs participate in liver regeneration either via intercellular junctions or a paracrine effect via ectovesicles though the direct evidence of relationship between TCs and hepatocytes/hepatic stem cells is required in the further studies. Further understanding the molecular and cellular mechanisms by which TCs affect hepatocytes proliferation and/or hepatic stem cells differentiation in liver regeneration will help establish novel therapeutic strategies for liver failure.

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Conflicts of interest

The authors declare there are no conflicts of interest.

References

1. Duncan AW, Soto-Gutierrez A. Liver repopulation and regeneration: new approaches to old questions. Curr Opin Organ Transplant. 2013; 18: 197–202.
2. Kandilis AN, Koskinas J, Tiniakos DG, et al. Liver regeneration: focus on cell types and topographic differences. Eur Surg Res Eur Chir Forsch Rech Chir Eur. 2010; 44: 1–12.
3. Itoh T, Miyajima A. Liver regeneration by stem/progenitor cells. Hepatology. 2014; 59: 1617–26.
4. Xiao J, Wang F, Liu Z, et al. Telocytes in liver: electron microscopy and immunofluorescent evidence. J Cell Mol Med. 2013; 17: 1537–42.
5. Popescu LM, Faussone-Pellegrini M-S. TELOCYTES - a case of serendipity: the widening way from Interstitial Cells of Cajal (ICC), via Interstitial Cajal-Like Cells (ICLC) to TELOCYTES. J Cell Mol Med. 2010; 14: 729–40.
6. Nicolescu MI, Bucur A, Dinca O, et al. Telocytes in parotid glands. Anat Rec. 2012; 295: 378–85.
7. Gherghiceanu M, Popescu LM. Cardiac telocytes - their junctions and functional implications. Cell Tissue Res. 2012; 348: 265–79.
8. Ceafalan L, Gherghiceanu M, Popescu LM, et al. Telocytes in human skin – are they involved in skin regeneration? J Cell Mol Med. 2012; 16: 1405–20.
9. Popescu BD, Gherghiceanu M, Kostin S, et al. Telocytes in meninges and choroid plexus. Neurosci Lett. 2012; 516: 265–9.
10. Cretoiu D, Cretoiu SM, Simionescu AA, et al. Telocytes, a distinct type of cell among the stromal cells present in the lamina propria of jejunum. Histol Histopathol. 2012; 27: 1067–78.
11. Luesma MJ, Gherghiceanu M, Popescu LM. Telocytes and stem cells in limbus and uvea of mouse eye. J Cell Mol Med. 2013; 17: 1016–24.
12. Gevaert T, De Vos R, Van Der Aa F, et al. Identification of telocytes in the upper lamina propria of the human urinary tract. J Cell Mol Med. 2012; 16: 2085–93.
13. Hatta K, Huang M-L, Weisel RD, et al. Culture of rat endometrial telocytes. J Cell Mol Med. 2012; 16: 1392–6.
14. Zheng Y, Zhu T, Lin M, et al. Telocytes in the urinary system. J Transl Med. 2012; 10: 188.
15. Corradi LS, Jesus MM, Fochi RA, et al. Structural and ultrastructural evidence for telocytes in prostate stroma. J Cell Mol Med. 2013; 17: 398–406.
16. Zhao B, Chen S, Liu J, et al. Cardiac telocytes were decreased during myocardial infarction and their therapeutic effects for ischaemic heart in rat. J Cell Mol Med. 2013; 17: 123–33.
17. Manetti M, Guiducci S, Ruffo M, et al. Evidence for progressive reduction and loss of telocytes in the dermal cellular network of systemic sclerosis. J Cell Mol Med. 2013; 17: 482–96.
18. Díaz-Flores L, Gutiérrez R, Sáez FJ, et al. Telocytes in neuromuscular spindles. J Cell Mol Med. 2013; 17: 457–68.
19. Yang Y, Sun W, Wu SM, et al. Telocytes in human heart valves. J Cell Mol Med. 2014; 18: 759–65.
20. Sheng J, Shim W, Lu J, et al. Electrophysiology of human cardiac atrial and ventricular telocytes. J Cell Mol Med. 2014; 18: 355–62.
21. Suciu L, Popescu LM, Gherghiceanu M, et al. Telocytes in human term placenta: morphology and phenotype. Cells Tissues Organs. 2010; 192: 325–39.
22. Popescu LM, Manole CG, Gherghiceanu M, et al. Telocytes in human epicardium. J Cell Mol Med. 2010; 14: 2085–93.
23. Gherghiceanu M, Manole CG, Popescu LM. Telocytes in endocardium: electron microscope evidence. J Cell Mol Med. 2010; 14: 2330–4.
24. Popescu LM, Gherghiceanu M, Suciu LC, et al. Telocytes and putative stem cells in the lungs: electron microscopy, electron tomography and laser scanning microscopy. Cell Tissue Res. 2011; 345: 391–403.
25. Popescu LM, Manole E, Serboiu CS, et al. Identification of telocytes in skeletal muscle interstitium: implication for muscle regeneration. J Cell Mol Med. 2011; 15: 1379–92.
26. Nicolescu MI, Popescu LM. Telocytes in the interstitium of human exocrine pancreas: ultrastructural evidence. *Pancreas*. 2012; 41: 949–56.

27. Cismasiu VB, Radu E, Popescu LM. Mir-193 expression differentiates telocytes from other stromal cells. *J Cell Mol Med*. 2011; 15: 1071–4.

28. Zheng Y, Zhang M, Qian M, et al. Genetic comparison of mouse lung telocytes with mesenchymal stem cells and fibroblasts. *J Cell Mol Med*. 2013; 17: 567–77.

29. Zheng Y, Cretoiu D, Yan G, et al. Comparative proteomic analysis of human lung telocytes with fibroblasts. *J Cell Mol Med*. 2014; 18: 568–89.

30. Sun XR, Zheng MH, Zhang MM, et al. Differences in the expression of chromosome 1 genes between lung telocytes and other cells: mesenchymal stem cells, fibroblasts, alveolar type II cells, airway epithelial cells and lymphocytes. *J Cell Mol Med*. 2014; 18: 801–10.

31. Manole CG, Cismașiu V, Gherghiceanu M, et al. Experimental acute myocardial infarction: telocytes involvement in neoangiogenesis. *J Cell Mol Med*. 2011; 15: 2284–96.

32. Bani D, Nistri S. New insights into the morphogenic role of stromal cells and their relevance for regenerative medicine: lessons from the heart. *J Cell Mol Med*. 2014; 18: 363–70.

33. Zhao B, Liao Z, Chen S, et al. Intramyocardial transplantation of cardiac telocytes decreases myocardial infarction and improves post-infarcted cardiac function in rats. *J Cell Mol Med*. 2014; 18: 780–89.

34. Vannucchi MG, Traini C, Manetti M, et al. Telocytes express PDGFRα in the human gastrointestinal tract. *J Cell Mol Med*. 2013; 17: 1099–108.

35. Gherghiceanu M, Popescu LM. Cardiomyocyte precursors and telocytes in epicardial stem cell niche: electron microscope images. *J Cell Mol Med*. 2010; 14: 871–7.

36. Suskind DL, Muench MO. Searching for common stem cells of the hepatic and hematopoietic systems in the human fetal liver: CD34+ cytokeratin 7/8+ cells express markers for stellate cells. *J Hepatol*. 2004; 40: 261–8.

37. Miyajima A, Tanaka M, Itoh I. Stem/progenitor cells in liver development, homeostasis, regeneration, and reprogramming. *Cell Stem Cell*. 2014; 14: 561–74.