Medical Molecular Morphology (2018) 51:199–207
https://doi.org/10.1007/s00795-018-0183-1

ORIGINAL PAPER

Hepatic stellate cells derived from the nestin-positive cells in septum transversum during rat liver development

Makoto Toi[1] · **Yoshihiro Hayashi**[2,3] · **Ichiro Murakami**[1,2]

Received: 15 December 2017 / Accepted: 24 January 2018 / Published online: 29 January 2018

Abstract

Hepatic stellate cells (HSCs) play a principal role in Vitamin A metabolism and are considered the major matrix-producing cell type in the diseased liver. Rat HSCs are identified by immunohistochemistry with myogenic or mesenchymal (desmin, vimentin, and alpha-smooth muscle actin) or neural (e.g., GFAP or neuronal cell adhesion molecule) markers. Embryonic origin of rat HSCs was determined using these markers. Nestin, an intermediate filament protein originally identified in neuronal stem or progenitor cells, is widely used as a stem cell marker, including hepatic stem cells in adult rat livers. Additionally, nestin is reportedly expressed in activated HSCs during liver injury and hepatic regeneration. However, little is known about nestin expression in rat fetal liver HSCs. The present study aimed to clarify nestin-positive HSC expression during rat liver development. At embryonic day (ED) 10.5, nestin expression in mesenchymal cells adjacent to the liver bud was detected by immunohistochemistry. At ED 11.5, nestin-positive cells were also detected in desmin-positive cells appearing and increasing in intensity by ED 16.5. However, nestin-positive cells in the parenchyma decreased by ED 20.5 or later. These findings reveal that the nestin-positive HSCs during rat liver development originate from nestin-positive mesenchymal cells in the septum transversum.

Keywords Hepatic stellate cell · Hepatogenesis · Nestin · Immunohistochemistry · Ultrastructure

Introduction

Hepatic stellate cells (HSCs), a mesenchymal cell type in the liver, are characterized by long cytoplasmic processes with Vitamin A containing fat droplets in the cytoplasm. HSCs are pericytes [1] of liver sinusoidal endothelial cells (SECs). Recent evidence suggests a role as a liver-resident antigen-presenting cell, presenting lipid antigens to and stimulating the proliferation of NK/T cells [2]. Their transformed myofibroblastic cells are involved in several liver diseases, liver fibrosis, and cirrhosis [3]. HSCs also have a relationship with hepatic angiomyolipoma as they share a similar gene expression profile [4].

✉ Ichiro Murakami
ichiro.murakami.09@gmail.com

[1] Department of Pathology, Kochi University Hospital, 185-1 Kohasu, Okoh, Nankoku, Kochi 783-8505, Japan

[2] Department of Pathology, School of Medicine, Kochi University, Kohasu, Okoh, Nankoku, Kochi 783-8505, Japan

[3] Equipment of Support Planning Office, Kochi University, Kohasu, Okoh, Nankoku, Kochi 783-8505, Japan

HSCs exhibit both mesenchymal and neuronal cell features by expressing vimentin and desmin, as well as glial fibrillary acidic protein (GFAP), neural cell adhesion molecule (N-CAM), synaptophysin, and nestin, respectively [5–9].

Cell migration is a central process in the development and maintenance of multicellular organisms [10]. Three cytoskeletal proteins, including intermediate filaments (IFs), microfilaments, and microtubules are critical regulators of cell migration and cell shape maintenance [11]. IFs are formed with 10-nm-diameter intracellular filaments and are subcategorized into five types based on similarities in amino acid sequences and protein structure [12].

Nestin, a Type VI intermediate filament, was first identified in the multipotent stem/progenitor cells of the central nervous system (CNS), and has been regarded as a marker of neuronal and non-neuronal stem/progenitor cells [13–15]. However, a large number of studies have confirmed nestin expression in several non-neuronal tissues during not only their developmental stages but also in conditions of injury or repair [14], which is widely expressed during development but is very rare in adult organs. Nestin has been detected

in several proliferating regions of the CNS and in developing mesenchyme and blood vessels during embryogenesis [16–18].

Subsequently, nestin was shown to be upregulated in stem cells of various organs, such as the pancreas [19, 20], testis [21], kidney [22], and liver [23]. Nestin expression generally decreased upon cellular differentiated. However, nestin expression is known to characterize mature cells and begin stem cells in a variety of linages. It can be induced during stem cell activation in response to liver [6], skeletal muscle [24] and the CNS [25] injuries.

In this study, we strived to determine by immunohistochemistry whether HSCs and another (myo)fibroblast derive from nestin-expressing mesenchymal stem cells during rat hepatogenesis. We discovered that a high expression of nestin was detectable in the mesenchymal cells between the liver bud and the septum transversum mesenchyme at ED 10.5. Nestin-expressing cells were also expressed in desmin, a rat HSC marker, during early hepatogenesis. However, desmin-positive HSCs decreased in nestin expression at ED 16.5 and later. At ED 20.5, nestin-positive cells were observed only in mesenchymal cells around the central veins and in the portal tracts. Our results suggest that nestin-expressing mesenchymal cells differentiate into HSCs, perivenular (myo) fibroblasts, and portal (myo)fibroblasts.

Materials and methods

Embryonic and adult normal liver

Pregnant rats of Wistar strain were purchased from SLC (Shizuoka, Japan). All animals were maintained under 12-h light/dark cycles with food and water available as desired.

Liver samples of embryos from adult rats were used at various times of gestation, beginning at ED 10.5. Embryos were removed from the uteri of mothers under ether anesthesia. A portion of the liver was immersed in 20% phosphate-buffered formaldehyde for immunohistochemical analysis, fixed in 4% paraformaldehyde (PFA) for immuno-electron microscopy and whole-mount immunohistochemistry or fixed in 2.5% glutaraldehyde for transmission electron microscopy. For RT-PCR examination, liver samples were immersed in RNA-later (Applied Biosystems, CA, USA) and stored at − 20 °C until use. Samples at all embryonic stages were prepared from at least three different litters.

Animal models of liver injury

Male Wistar rats, 200–250 g body weight, were used in the carbon tetrachloride (CCl_4) models. CCl_4 acute damaged liver was induced as described [26, 27]. Liver tissues were obtained 24 and 48 h after CCl_4 treatment. These samples were immersed in 20% phosphate-buffered formaldehyde for immunohistochemical staining.

Whole-mount immunohistochemistry

Each embryo was fixed in 4% PFA in phosphate-buffered saline (PBS) for 4 h and immersed in 100% methanol for 20 min at − 20 °C. The fixed samples were then bleached with 2% hydrogen peroxide in methanol for 1 h at room temperature to block endogenous peroxidase. The rehydrated specimens were first incubated with 1% normal rabbit serum in 0.1% TritonX-100 in PBS (PBST) consisting of 1% skim milk for 1 h at 4 °C, followed by incubation with anti-nestin antibody in PBST at 4 °C overnight. After washing eight times with PBST each for 30 min at 4 °C, the specimens were incubated with a peroxidase anti-mouse antibody (Simple Stain Nichirei, Tokyo, Japan) overnight at 4 °C. After unbinding the second antibody by washing with PBST, the specimens were incubated in 3,3′-diaminobenzidine tetrahydrochloride (DAB; Sigma, St. Louis, Mo. USA) and additionally hydrogen peroxidase to 0.02% (v/v) for 1 h. The enzymatic reaction was allowed to proceed until the desired color intensity was reached. The specimens were then rinsed three times in PBST. Specimens were photographed with a digital camera mounted on a microscope.

Immunohistochemical detection

Paraffin sections (3 μm thick) were cut and mounted on silane-coated glass slides. The deparaffinized sections were microwaved for 15 min in 10 mM citric acid (pH 6.0) for antigen retrieval. The sections were incubated with 10% normal rabbit serum in 0.01 M PBS for 10 min at room temperature, and then with each monoclonal antibody for anti-nestin (rat401 chemicon Carpinteria, CA, USA), desmin (D33, Dako, Glostrup Denmark; 1:50) with PBS containing 0.1% bovine serum albumin at 4 °C overnight. After washing with PBS, the sections were incubated with biotinylated rabbit anti-mouse immunoglobulin (Dako Glostrup Denmark; 1:200) for 1 h at room temperature, washed again with PBS, and incubated with avidin–biotin peroxidase complex (ABC; Vector Laboratories, Inc., Burlingame, CA, USA) solution. Finally, the sections were immersed in a substrate solution of DAB. As a control, sections were processed without incubation with the primary antibody.

Double immunofluorescence analysis

To identify which cells expressed nestin, a double immunofluorescence method was carried out for several markers: desmin (HSCs and smooth muscle cells). The sections were incubated with 10% normal rabbit serum in 0.01 M PBS for 10 min at room temperature, and then with each

monoclonal antibody for anti-nestin with PBS containing 0.1% bovine serum albumin (BSA) at 4 °C overnight. After washing with PBS, the sections were incubated with biotinylated rabbit anti-mouse immunoglobulin for 1 h at room temperature, washed with PBS, and incubated with FITC-labeled streptavidin (Invitrogen Corp., Carlsbad, CA, USA) solution. Immunolabeled sections were then incubated in 10 mM citric acid (pH 6.0) for 30 min at 95 °C for antigen deactival. Subsequently, the specimens were immersed in anti-desmin antibody at 4 °C overnight. After washing with PBS, the sections were treated with secondary antibody (biotin-labeled rabbit anti-mouse immunoglobulin) for 1 h, washed with PBS, and incubated with Texas Red-labeled streptavidin (Invitrogen Corp., Carlsbad, CA, USA). Finally, the slides were covered with vectashield mounting medium with DAPI (Vector Laboratories, UK) to visualize cellular nuclei. As a control, sections were processed without incubation with the primary antibody. Specimens were photographed with an Olympus digital camera (DP70, Olympus Corporation, Tokyo, Japan) mounted on an Olympus fluorescent microscope (BX50).

Electron and immuno-electron microscopy

Embryonic tissues fixed in 2.5% glutaraldehyde in 0.1 M PBS (pH 7.4) for 4 h at 4 °C were then post-fixed in 1% osmium tetroxide in PBS for 1 h at 4 °C. This was followed by dehydration and embedding in epoxy resin. To select optimal areas, semi-thin sections were stained with toluidine blue. Ultra-thin sections were stained with uranyl acetate and lead citrate then examined with a JEM 100S electron microscope (JEOL, Tokyo, Japan).

Whole fetuses (ED 12.5) were fixed in 4% PFA solution for 6 h. Following fixation, the specimens were incubated overnight with 10% sucrose in PBS. Frozen sections were cut into 10-μm slices and washed with PBS. The sections were pre-incubated with 10% normal rabbit serum for 30 min, and then incubated for 24 h with anti-nestin antibody at 4 °C. Sections were then incubated with biotinylated rabbit anti-mouse immunoglobulin overnight at 4 °C. Finally, the sections were incubated with ABC solution for 1 h at room temperature. The peroxidase reaction was developed by incubating sections with 0.02% DAB in 0.05 M Tris–HCl (pH 7.6) for 1 h at room temperature and then 0.02% DAB in 0.05 M Tris–HCl (pH 7.6) containing 0.01% H_2O_2 for 10 min. The tissue sections were post-fixed with 1% OsO_4 in a 0.1 M PBS (pH 7.4) for 1 h, dehydrated in a graded ethanol series, and covered with Epon812. Ultra-thin sections were observed with an electron microscope.

RT-PCR

Total RNA was extracted from frozen tissue using TRIzol reagent (Invitrogen, Corp., Carlsbad, CA) according to the manufacturer's instructions. First-strand cDNAs were synthesized with Oligo (dt) primer and the Superscript III first-strand synthesis system (Invitrogen, Corp., Carlsbad, CA). Each single-stranded cDNA was diluted for subsequent PCR amplification. Standard PCR procedure was performed in 15 microvolumes of PCR buffer. To detect rat nestin, the primers 5′-AACCACAGGAGTGGGAACTG-3′ (rat nestin forward) and 5′-TCTGGCATTGACTGAGCAAC-3′ (rat nestin reverse) were used. As a quantitative control, glyceraldehydrate-3-phosphate dehydrogenase (GAPDH) was amplified with primer 5′-CTCATGACCACAGTCCATGC-3′ and 5′-TTCAGCTCTGGGATGACCTT-3′. PCR conditions were an initial denaturation at 94 °C for 7 min, following by 30 cycles of 94 for 30 s, 55 for 1 min, and 72 for 1 min, and a final extension step of 72 for 10 min. Each PCR product (15 μg/μl) was visualized by ethidium bromide staining on 6% acrylamide gel.

Results

Analysis of nestin protein expression in rat embryos by whole-mount immunohistochemistry

At ED 11.5, whole-mount immunohistochemistry showed the expression of nestin in several regions. Strong staining was detected in the neural tube, the definitive gut endoderm, branchial arches, and liver bud (Fig. 1a). When examined at ED 12.5, the liver, somites, forelimb buds, and branchial arches were stained strongly (Fig. 1b).

Distribution of nestin-positive cells in developed rat liver

At ED 10.5, the ventral foregut endoderm started to develop and form a liver bud. Nestin was expressed in mesenchymal cells adjacent to the liver bud. Cell types such as neural epithelial cells, neural crest, and mesenchymal cells of somite were also positive for nestin (Fig. 2a, b). At ED 11.5, the nestin expression was detected in mesenchymal cells mixed with migrating hepatoblasts in septum transversum mesenchyme (Fig. 2c). From ED 12.5 to 16.5 strong expressions of nestin were observed in the sinusoidal cells, such as HSCs and SECs around the hepatic sinusoid (Fig. 2d, e). In the rat liver at ED 20.5, numbers of nestin-positive sinusoidal cells were markedly decreased in the liver parenchyma (Fig. 2f). The expression levels of nestin gradually decreased after this stage; however, mesenchymal cells around the central and portal veins continued to express nestin at high levels

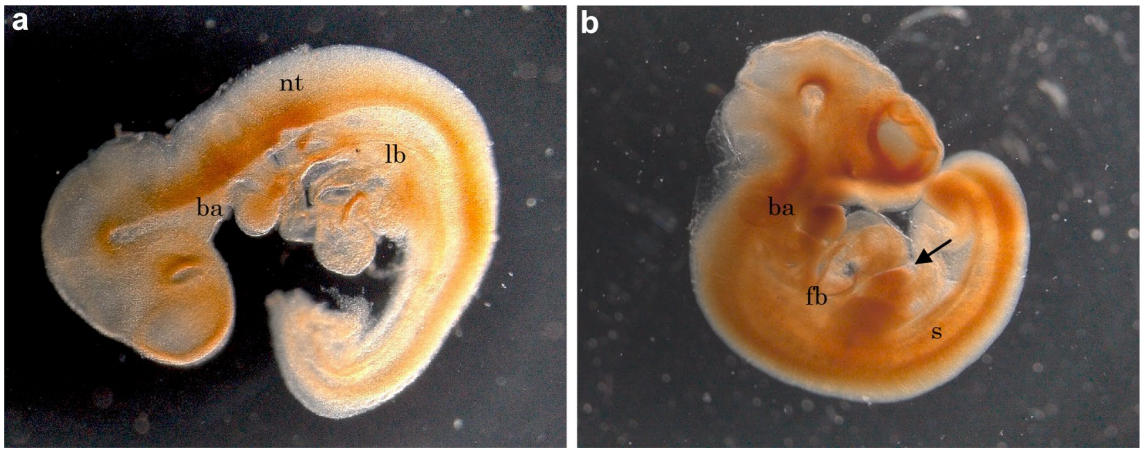

Fig. 1 At ED 11.5, whole-mount immunohistochemistry revealed strong nestin expression in the neural tube (nt), branchial arches (ba) and liver bud (lb) (**a**). At ED 12.5, the liver (arrow), somites (s), forelimb buds (fb) and branchial arches (ba) were strongly stained (**b**)

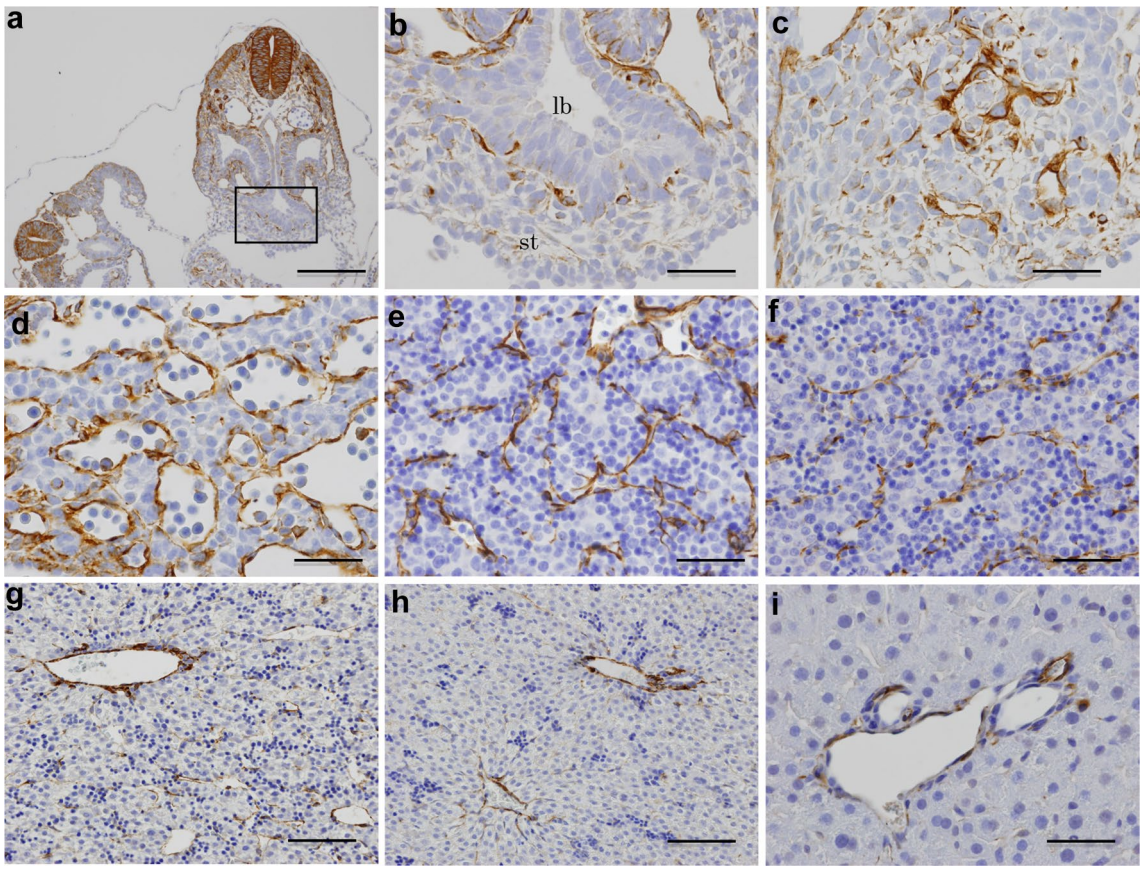

Fig. 2 At ED 10.5, nestin was expressed in neural epithelial cells, neural crest, and mesenchymal cells of somite (**a**). Higher magnification of boxed area in **a** (**b**). Nestin-positive cells were observed in mesenchymal cells around and adjacent to the liver bud (lb) in the septum transversum (st) (**c**). At ED 11.5, the nestin expression was detected in mesenchymal cells mixed with migrating hepatoblasts in the septum transversum. From ED 12.5 to 16.5 nestin expression was observed in the sinusoidal cells, such as hepatic stellate cells and sinusoidal endothelial cells around hepatic sinusoid [**d** (ED 12.5), **e** (ED 14.5), **f** (ED 16.5)]. In the rat liver at ED 20.5, numbers of nestin-positive sinusoidal cells were markedly decreased in the liver parenchyma (**g**). The expression levels of nestin gradually decreased after this stage; however, mesenchymal cells around the central and portal veins continued to express nestin at high levels [**h** (postnatal day 3)]. In the adult liver, the mesenchymal cells around the central and the portal vein, and periductular stromal cells adjacent to biliary epithelium were positive (**i**). Bars: 200 μm (**a**), 100 μm (**e**–**h**), 50 μm (**b, c, i**)

(Fig. 2g). In the postnatal liver, strong nestin-positive cells were observed around the portal vein and immature bile duct in the portal areas (Fig. 2h). In the adult liver, sinusoidal cells were negative for nestin, whereas the mesenchymal cells around the central and the portal vein and the periductular stromal cells adjacent to biliary epithelium were positive (Fig. 2i). Other cell types, such as hepatocytes and biliary epithelial cells, were not reactive with anti-nestin antibody.

Double immunofluorescence analysis

Double immunostaining for nestin and desmin was performed to examine whether sinusoidal cells were positive for desmin-expressed nestin during liver development. At ED 10.5, nestin-expressing cells were observed around the liver bud, and a small number of these cells expressed in desmin (Fig. 3**a**–**d**). At ED 11.5, many, but not all, nestin-positive cells were co-expressed desmin (Fig. 4**a**–**c**). At ED 14.5, a large number of desmin-positive cells existed in the liver parenchyma; however, desmin-positive HSCs were largely negative for nestin (Fig. 4**d**–**f**).

Ultrastructural finding of liver development

At an ultrastructural observation at ED 10.5, the liver bud contacted the mesenchymal cells in the septum transversum through cellular protrusions (Fig. 5a, b). At ED 11.5, immature SECs were encircled by immature mesenchymal cells and migrating hepatoblasts. They had narrow lumens without blood cells. Mesenchymal cells in septum transversum mesenchyme were stellate in shape, having many slender processes (Fig. 5c). Likewise, at ED 12.5, cell–cell adhesions between hepatoblasts were loose. Mesenchymal cells existed under the immature SECs. These cells had many cellular processes and contact with other mesenchymal cells (Fig. 5d).

By immuno-electron microscopy and using anti-nestin antibody, strong positive cells were detected as electron-dense deposits on the cytoplasm of HSCs under the SECs

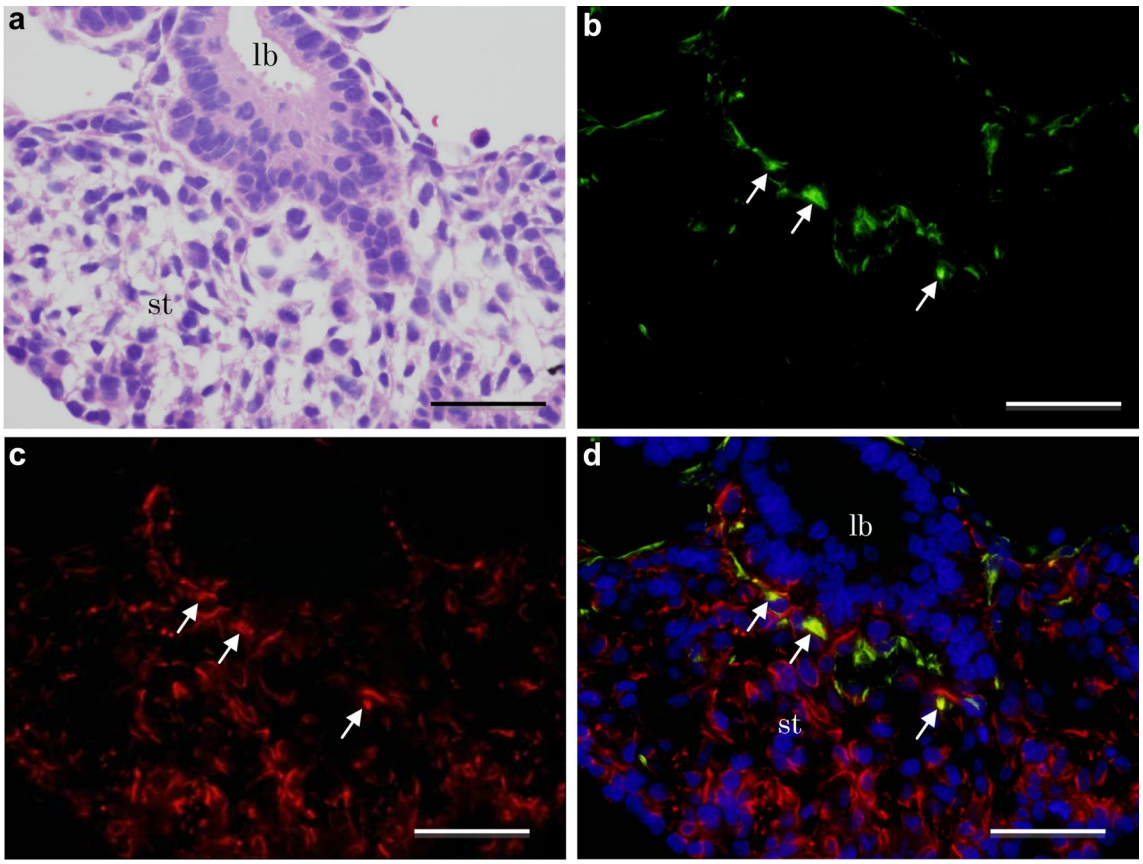

Fig. 3 Transverse section of an ED 10.5 embryo. Double immunostaining for nestin and desmin was performed to examine whether immature hepatic stellate cells (hsc) were positive for desmin-expressed nestin during liver development (**a**: H&E). Desmin was considered a marker for HSC. Nestin-expressing cells (**b, d**: green) were observed around the liver bud (lb) in the septum transversum (st). A small number of nestin-positive cells expressed desmin (**c, d**: red). Nestin and desmin double-positive cells were indicated by white arrows. Bars: 50 μm

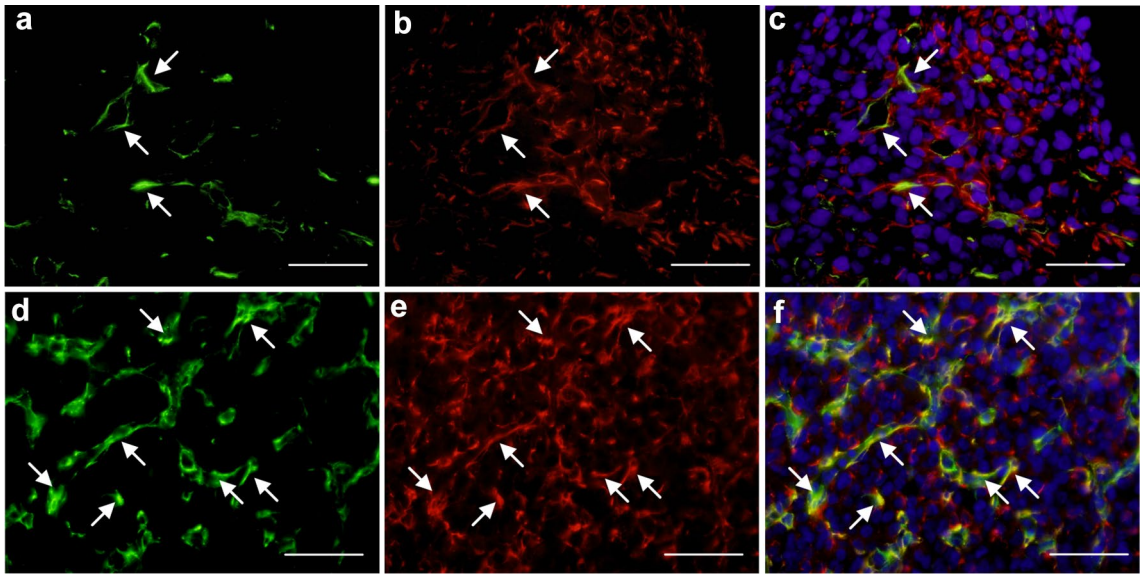

Fig. 4 At ED 11.5, a small number of nestin-positive cells co-expressed desmin (**a**–**c**). At ED 14.5, large numbers of nestin-positive cells existed in the liver parenchyma; however, desmin-positive hepatic stellate cells were largely negative for nestin (**d**–**f**). Arrows: both nestin- and desmin-positive hepatic stellate cells. Bars: 50 μm

at ED 12.5. More mature SECs were negative for nestin (Fig. 5e). Similarly, nestin-positive cells were identified as both SECs and HSCs in areas with many hematopoietic cells in early hepatogenesis (Fig. 5f).

Analysis of nestin mRNA expression in rat embryos and adult liver

Temporal expression profiles of nestin mRNA were examined by RT-PCR analysis. As shown in Fig. 6, the transcripts were detected throughout all embryonic stages from ED 13.5 to 20.5 and the adult liver. Nestin mRNA gradually decreased during liver development.

Distribution of nestin-positive cells in injured liver

Necrotic hepatocytes were observed around the central veins at 24 and 48 h after CCl_4 injection (Fig. 7). In this stage, nestin-positive cells were distributed in activated spindle-shaped HSCs (myofibroblast-like cells) around the pericentral areas. In addition, nestin-negative HSCs were existent around the necrotic tissue.

Discussion

Nestin, an intermediate filament protein, was first described as expressed in central nervous system stem cells [15], and later in several other tissues and cells, including cardiac myogenic cells [16], Leydig cells [28], and adrenocortical cells [29, 30]. Pancreatic stellate cells and vascular endothelial cells in the pancreas [20, 31–34], kidney [22, 35], and many other cells have been reported to contain nestin. In addition, nestin has been suggested to be expressed in activated HSCs in injured rat livers, not in quiescent HSCs in normal livers [6].

We analyzed the expression of nestin during the development of rat livers by immunohistochemistry from ED 10.5 to ED 20.5 as well as the adult normal and injured liver. The results showed that nestin-positive cells are expressed in the mesenchymal cells adjacent to liver bud at ED 10.5. In this stage, epithelial cells of the neural tube and mesenchymal cells of the neural crest and somite were positive for nestin. At ED 12.5–16.5, nestin-positive cells were detected in sinusoidal cells, both HSCs and SECs, in the liver parenchyma and in mesenchymal cells around the large blood vessel. Starting at ED 16.5, the intensity of nestin positivity in sinusoidal cells gradually decreased. In the adult normal rat liver, sinusoidal cells were negative for nestin, whereas the mesenchymal cells around the central veins, the portal vein, and bile duct epithelial cells were positive.

HSCs, a mesenchymal cell type in the liver, are characterized by long cytoplasmic processes with vitamin A containing fat droplets in the cytoplasm. HSCs are pericytes of liver sinusoidal SECs. Activation of HSCs into myofibroblastic cells is involved in several liver diseases, liver fibrosis, and cirrhosis. HSCs show features of both mesenchymal cells and neuronal cells by expressing vimentin, and desmin, as well as GFAP, N-CAM, synaptophysin, respectively [5–9]. In addition, nestin expression

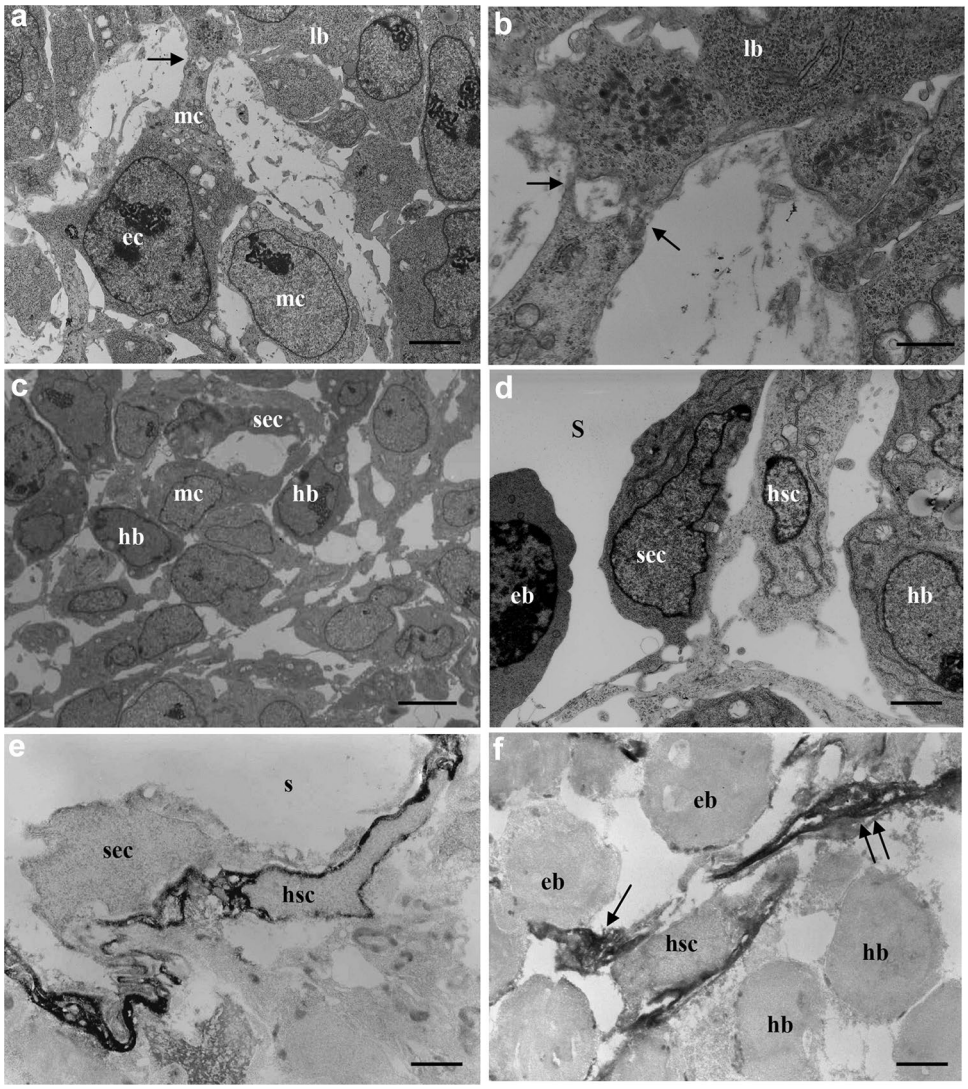

Fig. 5 Electron microscope. At ED 10.5, the liver bud (lb) contacted the mesenchymal cells (mc) in the septum transversum through cellular protrusions (arrows in **a, b**). At ED 11.5, immature sinusoidal endothelial cells (sec) were encircled by mesenchymal cells and migrating hepatoblasts (hb), and had narrow lumens without blood cells. Mesenchymal cells in the septum transversum were stellate in shape, having many slender processes (**c**). At ED 12.5, immature hepatic stellate cells (hsc) existed under the immature SECs, These cells had many cellular processes and contacts with other mesenchymal cells (**d**). Immuno-electron microscope using anti-nestin antibody at ED 12.5. Strong positive cells were detected as electron-dense deposits in the cytoplasm of immature HSC under the immature sinusoidal endothelial cells (**e**). Similarly, it is recognized that nestin-positive cells were both SECs and HSCs in areas with many hematopoietic cells (**f**). EB: erythroblasts; S: sinusoid; arrow: immature SEC; double arrow: HSC process; bars: 3 μm (**a, d–f**), 1 μm (**b**), 10 μm (**c**)

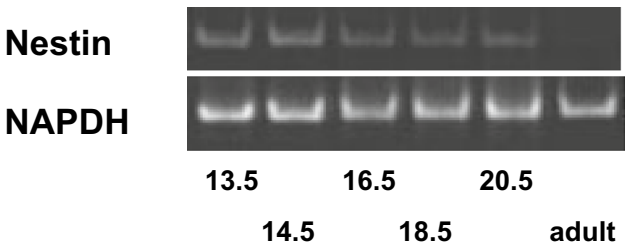

Fig. 6 Nestin mRNA was detected throughout all embryonic stages from ED 13.5 to 20.5 and in the adult liver. The expression of nestin gradually decreased during liver development

was detected in activated HSCs, myofibroblast-like cells, and in CCl4-induced liver fibrogenesis [6]. Our observation also showed that myofibroblast-like cells derived from HSCs were positive for nestin around the pericentral areas after acute liver injury with CCl_4.

On the other hand, the embryonic origin of HSCs in the liver has remained obscure. It has been postulated that the origin of HSCs is related to the mesenchymal lineage [36], the neuro-ectoderm [6, 37], the endoderm [38], or the mesothelial live capsule [39, 40]. However, Cassiman et al. reported that HSCs are not derived from the neural crest in transgenic mice expressing yellow fluorescent protein in all neural crest cells [39]. Furthermore, it was reported that bone marrow cells or hematopoietic stem cells become HSCs in the adult injured liver [41, 42]. Recently, HSCs were derived from the submesothelial cells beneath mesothelial cells in developing mouse liver [43, 44]. However, in rats there was no report that HSCs were derived from submesothelial cells. In our observation, submesothelial cells were positive for desmin, not nestin (data not shown) might be one of the origins of HSCs. In addition, HSCs in the embryonic or adult liver might be of a different origin.

In conclusion, we found that nestin, a Class VI intermediate filament protein, is transiently expressed in HSCs during

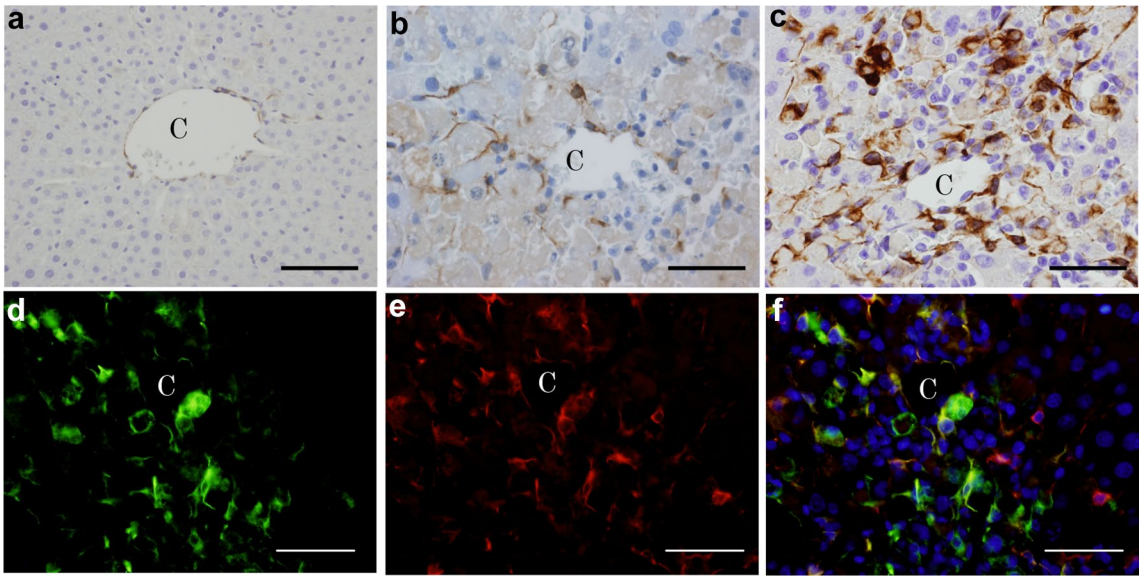

Fig. 7 Necrotic hepatocytes were observed around the central veins at 24 and 48 h after CCl_4 injection. In this stage, nestin-positive cells were distributed in activated spindle-shaped HSCs around pericentral areas at 24 h (**b**) and 48 h (**d–f**) after CCl_4 injection (**a** hepatocyte without CCl_4 injection as control). The majority of desmin-positive hepatic stellate cells were nestin positive; however, only nestin-positive cells were also observed in the necrotic areas. Nestin-negative HSCs were existent around the necrotic tissue. Bars: 100 μm (**a**), 50 μm (**b–f**) *c* central vein

rat hepatogenesis, but not in an adult normal liver. At ED 10.5, when the rat liver starts, these results may imply that nestin is a significant marker for undifferentiated and activated rat hepatic stellate cells.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflicts of interest.

References

1. Armulik A, Genove G, Betsholtz C (2011) Pericytes: developmental, physiological, and pathological perspectives, problems, and promises. Dev Cell 21:193–215
2. Winau F, Hegasy G, Weiskirchen R, Weber S, Cassan C, Sieling PA, Modlin RL, Liblau RS, Gressner AM, Kaufmann SH (2007) Ito cells are liver-resident antigen-presenting cells for activating T cell responses. Immunity 26:117–129
3. Birukawa NK, Murase K, Sato Y, Kosaka A, Yoneda A, Nishita H, Fujita R, Nishimura M, Ninomiya T, Kajiwara K, Miyazaki M, Nakashima Y, Ota S, Murakami Y, Tanaka Y, Minomi K, Tamura Y, Niitsu Y (2014) Activated hepatic stellate cells are dependent on self-collagen, cleaved by membrane type 1 matrix metalloproteinase for their growth. J Biol Chem 289:20209–20221.s
4. Kannangai R, Diehl AM, Sicklick J, Rojkind M, Thomas D, Torbenson M (2005) Hepatic angiomyolipoma and hepatic stellate cells share a similar gene expression profile. Hum Pathol 36:341–347
5. Niki T, De Bleser PJ, Xu G, Van Den Berg K, Wisse E, Geerts A (1996) Comparison of glial fibrillary acidic protein and desmin staining in normal and CCl4-induced fibrotic rat livers. Hepatology 23:1538–1545
6. Niki T, Pekny M, Hellemans K, Bleser PD, Berg KV, Vaeyens F, Quartier E, Schuit F, Geerts A (1999) Class VI intermediate filament protein nestin is induced during activation of rat hepatic stellate cells. Hepatology 29:520–527
7. Nakatani K, Seki S, Kawada N, Kobayashi K, Kaneda K (1996) Expression of neural cell adhesion molecule (N-CAM) in perisinusoidal stellate cells of the human liver. Cell Tissue Res 283:159–165
8. Enzan H, Himeno H, Iwamura S, Saibara T, Onishi S, Yamamoto Y, Hara H (1994) Immunohistochemical identification of Ito cells and their myofibroblastic transformation in adult human liver. Virchows Arch 424:249–256
9. Cassiman D, van Pelt J, De Vos R, Van Lommel F, Desmet V, Yap SH, Roskams T (1999) Synaptophysin: a novel marker for human and rat hepatic stellate cells. Am J Pathol 155:1831–1839
10. Franz CM, Jones GE, Ridley AJ (2002) Cell migration in development and disease. Dev Cell 2:153–158
11. Tang DD, Gerlach BD (2017) The roles and regulation of the actin cytoskeleton, intermediate filaments and microtubules in smooth muscle cell migration. Respir Res 18:54
12. Herrmann H, Bar H, Kreplak L, Strelkov SV, Aebi U (2007) Intermediate filaments: from cell architecture to nanomechanics. Nat Rev Mol Cell Biol 8:562–573
13. Dahlstrand J, Collins VP, Lendahl U (1992) Expression of the class VI intermediate filament nestin in human central nervous system tumors. Cancer Res 52:5334–5341
14. Wiese C, Rolletschek A, Kania G, Blyszczuk P, Tarasov KV, Tarasova Y, Wersto RP, Boheler KR, Wobus AM (2004) Nestin expression–a property of multi-lineage progenitor cells? Cell Mol Life Sci 61:2510–2522
15. Lendahl U, Zimmerman LB, McKay RD (1990) CNS stem cells express a new class of intermediate filament protein. Cell 60:585–595

16. Kachinsky AM, Dominov JA, Miller JB (1995) Intermediate filaments in cardiac myogenesis: nestin in the developing mouse heart. J Histochem Cytochem 43:843–847
17. Mokry J, Nemecek S (1999) Cerebral angiogenesis shows nestin expression in endothelial cells. Gen Physiol Biophys 18(Suppl 1):25–29
18. Yang J, Bian W, Gao X, Chen L, Jing N (2000) Nestin expression during mouse eye and lens development. Mech Dev 94:287–291
19. Lumelsky N, Blondel O, Laeng P, Velasco I, Ravin R, McKay R (2001) Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. Science 292:1389–1394
20. Esni F, Stoffers DA, Takeuchi T, Leach SD (2004) Origin of exocrine pancreatic cells from nestin-positive precursors in developing mouse pancreas. Mech Dev 121:15–25
21. Frojdman K, Pelliniemi LJ, Lendahl U, Virtanen I, Eriksson JE (1997) The intermediate filament protein nestin occurs transiently in differentiating testis of rat and mouse. Differentiation 61:243–249
22. Bertelli E, Regoli M, Fonzi L, Occhini R, Mannucci S, Ermini L, Toti P (2007) Nestin expression in adult and developing human kidney. J Histochem Cytochem 55:411–421
23. Gleiberman AS, Encinas JM, Mignone JL, Michurina T, Rosenfeld MG, Enikolopov G (2005) Expression of nestin-green fluorescent protein transgene marks oval cells in the adult liver. Dev Dyn 234:413–421
24. Vaittinen S, Lukka R, Sahlgren C, Hurme T, Rantanen J, Lendahl U, Eriksson JE, Kalimo H (2001) The expression of intermediate filament protein nestin as related to vimentin and desmin in regenerating skeletal muscle. J Neuropathol Exp Neurol 60:588–597
25. Clarke SR, Shetty AK, Bradley JL, Turner DA (1994) Reactive astrocytes express the embryonic intermediate neurofilament nestin. Neuroreport 5:1885–1888
26. Kawai S, Enzan H, Hayashi Y, Jin YL, Guo LM, Miyazaki E, Toi M, Kuroda N, Hiroi M, Saibara T, Nakayama H (2003) Vinculin: a novel marker for quiescent and activated hepatic stellate cells in human and rat livers. Virchows Arch 443:78–86
27. Tao LH, Enzan H, Hayashi Y, Miyazaki E, Saibara T, Hiroi M, Toi M, Kuroda N, Naruse K, Jin YL, Guo LM (2000) Appearance of denuded hepatic stellate cells and their subsequent myofibroblast-like transformation during the early stage of biliary fibrosis in the rat. Med Electron Microsc 33:217–230
28. Lobo MV, Arenas MI, Alonso FJ, Gomez G, Bazan E, Paino CL, Fernandez E, Fraile B, Paniagua R, Moyano A, Caso E (2004) Nestin, a neuroectodermal stem cell marker molecule, is expressed in Leydig cells of the human testis and in some specific cell types from human testicular tumours. Cell Tissue Res 316:369–376
29. Bertelli E, Regoli M, Lucattelli M, Bastianini A, Fonzi L (2002) Nestin expression in rat adrenal gland. Histochem Cell Biol 117:371–377
30. Toti P, Regoli M, Nesi G, Occhini R, Bartolommei S, Fonzi L, Bertelli E (2005) Nestin expression in normal adrenal gland and adrenocortical tumors. Histol Histopathol 20:1115–1120
31. Lardon J, Rooman I, Bouwens L (2002) Nestin expression in pancreatic stellate cells and angiogenic endothelial cells. Histochem Cell Biol 117:535–540
32. Delacour A, Nepote V, Trumpp A, Herrera PL (2004) Nestin expression in pancreatic exocrine cell lineages. Mech Dev 121:3–14
33. Selander L, Edlund H (2002) Nestin is expressed in mesenchymal and not epithelial cells of the developing mouse pancreas. Mech Dev 113:189–192
34. Bernardo AS, Barrow J, Hay CW, McCreath K, Kind AJ, Schnieke AE, Colman A, Hart AW, Docherty K (2006) Presence of endocrine and exocrine markers in EGFP-positive cells from the developing pancreas of a nestin/EGFP mouse. Mol Cell Endocrinol 253:14–21
35. Chen J, Boyle S, Zhao M, Su W, Takahashi K, Davis L, Decaestecker M, Takahashi T, Breyer MD, Hao CM (2006) Differential expression of the intermediate filament protein nestin during renal development and its localization in adult podocytes. J Am Soc Nephrol 17:1283–1291
36. Enzan H, Himeno H, Hiroi M, Kiyoku H, Saibara T, Onishi S (1997) Development of hepatic sinusoidal structure with special reference to the Ito cells. Microsc Res Tech 39:336–349
37. Eng FJ, Friedman SL (2000) Fibrogenesis I. New insights into hepatic stellate cell activation: the simple becomes complex. Am J Physiol Gastrointest Liver Physiol 279:G7–G11
38. Kiassov AP, Van Eyken P, van Pelt JF, Depla E, Fevery J, Desmet VJ, Yap SH (1995) Desmin expressing nonhematopoietic liver cells during rat liver development: an immunohistochemical and morphometric study. Differentiation 59:253–258
39. Cassiman D, Barlow A, Vander Borght S, Libbrecht L, Pachnis V (2006) Hepatic stellate cells do not derive from the neural crest. J Hepatol 44:1098–1104
40. Geerts A (2004) On the origin of stellate cells: mesodermal, endodermal or neuro-ectodermal? J Hepatol 40:331–334
41. Baba S, Fujii H, Hirose T, Yasuchika K, Azuma H, Hoppo T, Naito M, Machimoto T, Ikai I (2004) Commitment of bone marrow cells to hepatic stellate cells in mouse. J Hepatol 40:255–260
42. Miyata E, Masuya M, Yoshida S, Nakamura S, Kato K, Sugimoto Y, Shibasaki T, Yamamura K, Ohishi K, Nishii K, Ishikawa F, Shiku H, Katayama N (2008) Hematopoietic origin of hepatic stellate cells in the adult liver. Blood 111:2427–2435
43. Asahina K, Tsai SY, Li P, Ishii M, Maxson RE, Sucov HM, Tsukamoto H (2009) Mesenchyma origin of hepatic stellate cells, submesothelial cells, and perivascular mesenchymal cells during mouse liver development. Hepatology 49:998–1011
44. Asahina K, Zhou B, Pu WT, Tsukamoto H (2011) Septum transversum-derived mesothelium gives rise to hepatic stellate cells and perivascular mesenchymal cells in developing mouse liver. Hepatology 53:983–995