

# Isolation of dendritic-cell-like S100 $\beta$ -positive cells in rat anterior pituitary gland

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**Abstract** S100 $\beta$ -protein-positive cells in the anterior pituitary gland appear to possess multifunctional properties. Because of their pleiotropic features, S100 $\beta$ -positive cells are assumed to be of a heterogeneous or even a non-pituitary origin. The observation of various markers has allowed these cells to be classified into populations such as stem/progenitor cells, epithelial cells, astrocytes and dendritic cells. The isolation and characterization of each heterogeneous population is a prerequisite for clarifying the functional character and origin of the cells. We attempt to isolate two of the subpopulations of

S100 $\beta$ -positive cells from the anterior lobe. First, from transgenic rats that express green fluorescent protein (GFP) driven by the S100 $\beta$  protein promoter, we fractionate GFP-positive cells with a cell sorter and culture them so that they can interact with laminin, a component of the extracellular matrix. We observe that one morphological type of GFP-positive cells possesses extended cytoplasmic processes and shows high adhesiveness to laminin (process type), whereas the other is round in shape and exhibits low adherence to laminin (round type). We successfully isolate cells of the round type from the cultured GFP-positive cells by taking advantage of their low affinity to laminin and then measure mRNA levels of the two cell types by real-time polymerase chain reaction. The resultant data show that the process type expresses vimentin (mesenchymal cell marker) and glial fibrillary acidic protein (astrocyte marker). The round type expresses dendritic cell markers, CD11b and interleukin-6. Thus, we found a method for isolating dendritic-cell-like S100 $\beta$ -positive cells by means of their property of adhering to laminin.

The authors have no conflicts of interest that might prejudice the impartiality of this research and have nothing to declare.

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## Introduction

The anterior pituitary (adenohypophysis) consists of the anterior and the intermediate lobes. The anterior lobe is composed of five types of hormone-producing cells, S100 $\beta$ -protein-positive cells and fenestrated sinusoids (i.e., endothelial cells and pericytes). S100 $\beta$ -positive cells are characterized by a star-like appearance and have the ability to make cell clusters that form a central lumen (Soji and Herbert 1989). Based on these histological features, S100 $\beta$ -positive cells in the anterior

lobe are commonly referred to as folliculo-stellate cells (Vila-Porcile 1972). Accumulating evidence indicates that S100 $\beta$ -positive cells have numerous functions; they are reported to act as stem cells, phagocytes and cells that regulate hormone release (Inoue et al. 1999; Allaerts and Vankelecom 2005). In addition, the gap junctions between them facilitate long-distance intra-pituitary communication (Soji and Herbert 1989; Fauquier et al. 2001). S100 $\beta$ -positive cells are heterogeneous with respect to their morphological and physiological characteristics. The functional heterogeneity of these cells has been studied by several groups who have found various gene expression profiles within an S100 $\beta$ -positive cell population, allowing the cells to be categorized into three subpopulations (Inoue et al. 1999; Allaerts and Vankelecom 2005). The first group produces glial fibrillary acidic protein (GFAP) and/or vimentin (astrocyte-like classic S100 $\beta$ -positive cells). The second group produces keratin (epithelial-cell-like classic S100 $\beta$ -positive cells) and the third group secretes interleukin-6 (dendritic-cell-like; Höfler 1984; Tachibana and Yamashima 1988; Allaerts et al. 1996). In addition to these data, S100 $\beta$ -positive cells have been proposed to be even more heterogeneous and partially to share stem/progenitor cell characteristics in the fetal and adult rat anterior pituitary (Yoshimura et al. 1977; Horvath and Kovacs 2002); recent immunohistochemical studies (Yoshida et al. 2009, 2011, 2013; Chen et al. 2013) suggest that these cells should be classified as having a fourth subpopulation. However, no method presently exists for isolating the heterogeneous S100 $\beta$ -positive cells that show characteristics of stem/progenitors or have astrocyte-like, epithelial cell-like, or dendritic-like cells in vivo. The separation and characterization of the heterogeneous S100 $\beta$ -positive cells are important for understanding their functions and their origin.

Recently, Itakura et al. (2007) succeeded in generating a transgenic rat strain that expresses green fluorescent protein (GFP) driven by an S100 $\beta$ -promoter specifically in S100 $\beta$ -positive cells. Using the anterior lobes of this S100 $\beta$ -GFP rat strain, we obtained mostly pure GFP-positive S100 $\beta$ -positive cell fractions with a cell sorter and were able to grow them in primary culture (Horiguchi et al. 2010). Previously, we observed that fractionated GFP-positive cells can be grouped into two types by their morphological changes under the influence of the extracellular matrix (ECM) during primary culture (Horiguchi et al. 2010). One shows flattened and extended cytoplasmic processes (process type of S100 $\beta$ -positive cells) and the other is round in shape (round type). Thus, these two types display different affinities to ECM. Hence, the present study was conducted to separate the round type from the process type and to examine the gene expression and colocalization of several molecules postulated as being characteristic of S100 $\beta$ -positive cells. We found that the round type expresses CD11b and

interleukin-6 (IL-6), which are characteristic of dendritic cells.

## Materials and methods

### Animals

The S100 $\beta$ -GFP transgenic Wistar-crlj strain rat was generated by integrating the reporter gene GFP driven by the rat S100 $\beta$  promoter (Itakura et al. 2007). Male rats aged 8–10 weeks and weighing 250–300 g were given access to food and water *ad libitum* and were housed under conditions of 12 h light and 12 h darkness. The rats were killed by exsanguination from the right atrium under deep Nembutal anesthesia and were then perfused with Hanks' balanced salt solution (Life Technologies, Palo Alto, Calif., USA) in order to obtain cells for primary culture. The present study was approved by the Institutional Animal Care and Use Committee of the School of Agriculture, Meiji University and by Jichi Medical University, based on the NIH Guidelines for the Care and Use of Laboratory Animals.

### Cell culture

Anterior lobes of S100 $\beta$ -GFP male rats were dispersed as described previously (Horiguchi et al. 2010). Dispersed cells were plated onto 8-well glass chamber slides (1 cm<sup>2</sup>/well; Nalge Nunc International, Rochester, N.Y., USA) coated with or without 10  $\mu$ g/cm<sup>2</sup> of laminin (Millipore, Bedford, Mass., USA), which is an ECM component of the basement membrane, at a density of  $1 \times 10^5$  cells/cm<sup>2</sup> in 400  $\mu$ l Medium 199 with Earle's salts (Life Technologies), supplemented with 10 % fetal bovine serum (Sigma-Aldrich, St. Louis, Mo., USA), 0.5 U/ml penicillin and 0.5  $\mu$ g/ml streptomycin (Life Technologies). Cells were then cultured for 72 h at 37 °C in a humidified atmosphere of 5 % CO<sub>2</sub> and 95 % air. We had previously observed that the morphological and functional changes of S100 $\beta$ -positive cells were not induced by 10 % fetal bovine serum for 72 h in primary culture (Horiguchi et al. 2010, 2011).

### Isolation of S100 $\beta$ -positive cells

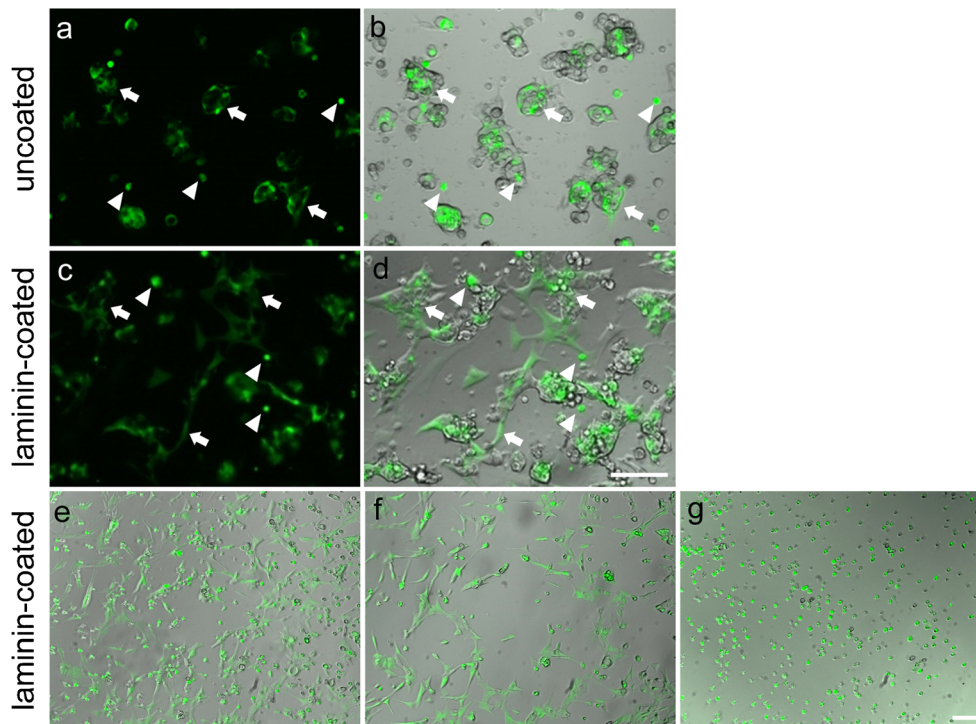
Dispersed cells of male S100 $\beta$ -GFP rats were separated into GFP-positive and GFP-negative cells by a cell sorter (MoFlo XDP; Beckman Coulter, Fullerton, Calif., USA) as described previously (Horiguchi et al. 2011). GFP-positive cells were plated onto 8-well glass chamber slides as described above. The GFP-positive cells were cultured for 24 h. The majority of GFP-positive cells showed flattened and markedly extended cytoplasmic processes (process type) but a small number of GFP-positive cells retained their round shape (round type).

**Table 1** Sequences of the specific primers for the studied genes

Genes (protein)	Primer sequence (5'→3')	Product size	Genbank accession number
<i>Vim</i> (vimentin)	Forward: TGCCCTGAAGCTGCTAACT Reverse: AGCAGGTCCTGGTATTCACG	108	NM_031140
<i>Gfap</i> (glial fibrillary acidic protein)	Forward: GGTGTGGAGTGCCTTCGTAT Reverse: TACGATGCCTGGGAAAAGG	122	NM_017009
<i>Cav3</i> (caveolin 3)	Forward: GGTGAACAGAGACCCCAAGA Reverse: ACGCCATCGAAGCTGTAAGT	99	NM_019155
<i>Bgn</i> (biglycan)	Forward: TCTCCAGGCTGCTTTCTGTT Reverse: GGGAGATCAAGTGCCAGGTA	102	NM_017087
<i>Cd11b</i> (integrin $\alpha$ M)	Forward: CCAGTGTGACATCCCTTCCT Reverse: AGTGCTCACAAAGCAGGAGGT	118	NM_012711
<i>Il6</i> (interleukin-6)	Forward: GCCAGAGTCATTCAGAGCAA Reverse: GAGCATTGGAAGTTGGGGTA	103	NM_012589
<i>Mmp14</i> (matrix metalloprotease 14)	Forward: AAGCTTGGCTGCAGCAGTAT Reverse: GCCTTGCCTGTCACCTTGTA	133	NM_031056
<i>Itgb6</i> (integrin $\beta$ 6)	Forward: AAAAAGACTGCCCAAACCT Reverse: TTCCGGTCGTGAAAGGATAC	124	NM_001004263
<i>b-Actin</i> ( $\beta$ -actin)	Forward: CATTGCTGACAGGATGCAGAAAG Reverse: TGCTGGAAGGTGGACAGTGAGG	138	NM_031144

The round type was removed from the dish with gentle pipetting and 400  $\mu$ l new medium was added to the remaining cells,

after the suspended media had been retrieved. The round cell type was collected by centrifuge from the retrieved medium



**Fig. 1** Fluorescence microscopy of primary cultured cells prepared from the anterior lobe of S100 $\beta$ -green fluorescent protein (GFP) transgenic rat. **a–d** Cells in anterior lobes of S100 $\beta$ -GFP rats were dispersed and cultured on an uncoated surface (**a, b**) or a laminin-coated surface (**c, d**). GFP images of S100 $\beta$ -positive cells were taken after a 72-h incubation in primary culture (**a, c**). GFP images were superimposed on phase-contrast images (**b, d**). S100 $\beta$ -positive cells (**a, c**, arrows) were flattened and had markedly extended cytoplasmic processes (process type). S100 $\beta$ -

positive cells displayed a round shape (round type; **a, c**, arrowheads). **e–g** GFP-positive cells were isolated with a cell sorter and cultured on a laminin surface. GFP images were superimposed on phase-contrast images. **e** Fractionated GFP-positive cells after a 24-h incubation. **f** The same field as in **e** taken 2 h after removal of round type S100 $\beta$ -positive cells by pipetting. **g** Collected round type S100 $\beta$ -positive cells replated onto a laminin-coated surface and incubated for 2 h. Bars 100  $\mu$ m

and cultured again for 24 h on laminin-coated 8-well glass. Cells were then cultured for a total of 48 h at 37 °C in a humidified atmosphere of 5 % CO<sub>2</sub> and 95 % air.

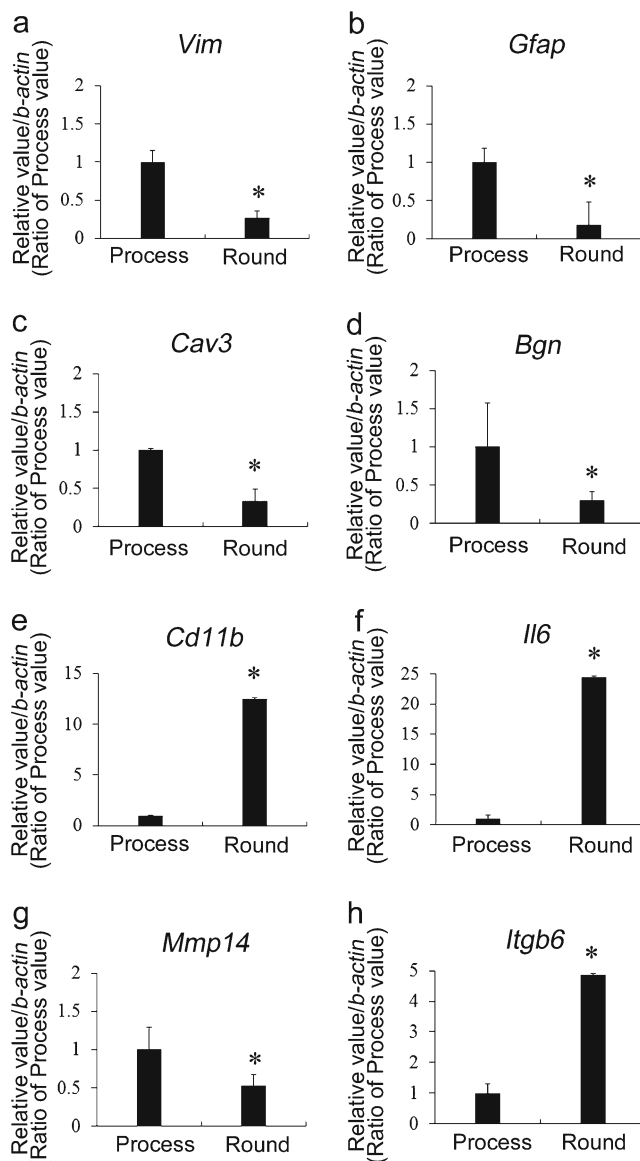
#### Quantification of mRNA levels by real-time polymerase chain reaction

Real-time polymerase chain reaction (PCR) was performed as described previously (Horiguchi et al. 2011). With Trizol (Life Technologies), total RNA fractions were prepared from the anterior lobe or the 48-h-cultured cells and then incubated with RNase-free DNase I (1 U/tube; Promega, Madison, Wis., USA) for 10 min at 37 °C. Subsequently, cDNA was synthesized by using the PrimeScript RT reagent kit (Takara, Otsu, Japan) with oligo-(dT)<sub>20</sub> primer (Invitrogen). Quantitative real-time PCR (ABI PRISM 7500HT; Applied Biosystems, Carlsbad, Calif., USA) was performed by using gene-specific primers and SYBR Premix Ex Taq (Takara) containing SYBR Green I. The sequences of the gene-specific primers are listed in Table 1. For normalization, we also quantified  $\beta$ -actin (*b-actin*). Relative quantification was conducted by using the standard curve method and was performed in at least three independent experiments.

#### In situ hybridization and immunohistochemistry

S100 $\beta$ -GFP male rats were perfused through the left ventricle with 4 % formaldehyde in 0.05 M phosphate buffer (pH 7.4) for 5 min under deep Nembutal anesthesia. The pituitary glands were then excised and immersed in the same fixative for 24 h at 4 °C, after which time the tissues were immersed for at least 2 days in a phosphate buffer containing 30 % sucrose at 4 °C. In situ hybridization was performed with digoxigenin (DIG)-labeled cRNA probes, as described in our previous report (Fujiwara et al. 2007a). The following DNA fragments were amplified from the rat pituitary cDNA library by PCR with specific primer sets: matrix metalloprotease 14 (*Mmp14*; GenBank accession no. NM\_022177), 5'-GTACCCCAAGTCAGCTCTGC-3' and 5'-GCCTTTGCCTGTAAGGTCAG-3' (product length 584 bp); interleukin-6 (*Il6*; NM\_012589), 5'-GCCAGAGT CATTCAAGCAA-3' and 5'-TGCAAGAAACCATCTG GCTA-3' (526 bp); integrin  $\alpha$ M (*Cd11b*; NM\_012711), 5'-CACTTTGGTGGTGGGAGACT-3' and 5'-GACAGGGA TCCAGAAGACCA-3' (553 bp); integrin  $\beta$ 6 (*Itgb6*; NM\_022205), 5'-AGGCTGTCTGTGGAGATA-3' and 5'-TTCCGGTCGTGAAAGGATAC-3' (573 bp). Amplified cDNA fragments were ligated into the pGEM-T vector (Promega) and cloned. Gene-specific antisense and sense DIG-labeled cRNA probes were made by means of the Roche DIG RNA Labeling Kit (Roche Diagnostics, Penzberg, Germany). A cryostat was used to obtain frozen sections (8  $\mu$ m in thickness), which were then mounted on glass slides.

Hybridization of the DIG-labeled cRNA probe was performed at 55 °C for 16 h. Visualization of each type of mRNA was carried out with alkaline-phosphatase-conjugated anti-DIG antibody (Roche Diagnostics) by using 4-nitroblue tetrazolium chloride (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP; Roche Diagnostics). For double-staining, after *Mmp14* or *Itgb6* mRNA had been detected by in situ hybridization, the section was immunostained as described in our previous report (Fujiwara et al. 2007b) by incubation



**Fig. 2** Comparison of mRNA levels between the process and round type S100 $\beta$ -positive cells. Expression levels of *Vim* (a), *Gfap* (b), *Cav3* (c), *Bgn* (d), *Cd11b* (e), *Il6* (f), *Mmp14* (g) and *Itgb6* (h) mRNA were determined by real-time PCR and normalized with an internal control (*b-actin*). Their mRNA levels were each calculated as ratios to the value of the process type. Expression levels of *Vim*, *Gfap*, *Cav3*, *Bgn* and *Mmp14* mRNA were higher in the process type (Process). On the other hand, those of *Cd11b* (e), *Il6* (f) and *Itgb6* (g) were higher in the round type (Round). Means $\pm$ SEM, n=6-8, data are from 3-4 cultures; \**P* <0.05. For an explanation of gene names, see Table 1



overnight at room temperature in phosphate-buffered saline (PBS) with primary antibodies. Primary antibodies against the following proteins were used for immunostaining: adrenocorticotropic hormone (ACTH), growth hormone (GH), prolactin (PRL), thyroid-stimulating hormone  $\beta$ -subunit (TSH $\beta$ ), luteinizing hormone  $\beta$ -subunit (LH $\beta$ ) and S100 protein, as reported previously (Fujiwara et al. 2007b), plus desmin (Nehls et al. 1992; 1:1200; Abcam, Tokyo, Japan). The absence of an observable non-specific reaction with normal rabbit serum was confirmed (data not shown). After being washed with PBS, the sections were incubated in PBS with biotinylated anti-rabbit IgG (Vector Laboratories, Burlingame, Calif., USA) for 30 min at 30 °C. The ABC method (Vector Laboratories) was performed with 3,3'-diaminobenzidine (Dojindo Laboratories, Kumamoto, Japan) as the substrate.

#### Double-fluorescence immunohistochemistry

Sections from S100 $\beta$ -GFP male rats were incubated in PBS containing 2 % normal goat serum for 20 min at 30 °C, then incubated overnight with mouse anti-rat vimentin monoclonal antibody (1:800, Dako, Glostrup, Denmark) and rabbit anti-rat GFAP polyclonal antibody (1:400, Dako) at room temperature. After being washed with PBS, the sections were incubated in PBS with Alexa-Fluor-568-conjugated goat anti-rabbit IgG and Alexa-Fluor-633-conjugated goat anti-mouse IgG (Life Technologies), diluted to 1:200 with PBS and washed with PBS again. The sections were scanned by using a confocal laser microscope (FV500, Olympus, Tokyo, Japan).

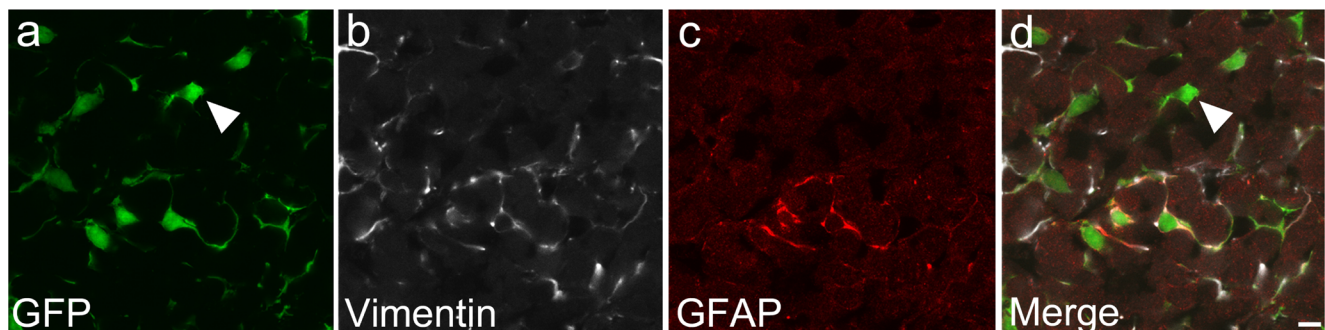
#### Statistical analysis

All data are presented as means $\pm$ SEM ( $n=6-8$ ; data from 3–4 cultures). Significant differences between groups were determined by the unpaired two-tailed Student's *t*-test. *P* values less than 0.05 were considered statistically significant.

## Results and discussion

S100 $\beta$ -GFP transgenic rats allowed us to distinguish living S100 $\beta$ -positive cells from GFP-negative cells, including the hormone-producing cells in the anterior pituitary gland. By using purified GFP-positive cells from the anterior lobes of S100 $\beta$ -GFP rats, we found that round S100 $\beta$ -positive cells appeared individually (Fig. 1a, b, arrowheads), whereas GFP-positive cells showed extended cytoplasmic processes that constructed aggregates by encircling hormone-producing cells (Fig. 1a, b, arrows). We further observed that the morphological difference in the GFP-positive cells were more prominent in primary cultures on a laminin surface (Fig. 1c, d). After a 72-h culture on a laminin surface, a small number of GFP-positive cells retained their round shape (round type, Fig. 1c, d, arrowheads), whereas the majority of GFP-positive cells had flattened out and had markedly extended cytoplasmic processes (process type, Fig. 1c, d, arrows). In our previous report, morphological changes to the process type under the influence of ECM were transduced by a caveolin-3-mediated integrin  $\beta$ 1 signaling pathway (Horiguchi et al. 2011). Caveolin 3 is a membrane protein that binds cholesterol and a number of signaling molecules that interact with integrin  $\beta$ 1 (Echarri et al. 2007). We suggest that this caveolin signaling plays only a minor role in round S100 $\beta$ -positive cells.

Next, we attempted to separate S100 $\beta$ -positive cells into the round and process types. At the first step, we collected the S100 $\beta$ -positive cells by means of GFP fluorescence with a cell sorter and cultured them on a laminin surface for 24 h (Fig. 1e). At the second step, the round type cells were isolated from the cultured cells by gentle pipetting, which peeled off most of the round types because of their low adhesiveness to the laminin. The majority of the remaining cells were of the process type (Fig. 1f). The round type cells were collected by centrifuge from the recovered medium and cultured on laminin-coated 8-well glass chamber slides (Fig. 1g). After another 24 h of cultivation, the round types retained their round shape on the laminin-coated surface (data not shown),



**Fig. 3** Observation of the process and round type S100 $\beta$ -positive cells in anterior lobe. **a-d** Cryosection from S100 $\beta$ -GFP rat showing GFP-positive cells in the anterior lobe (**a**, green) were immunostained for vimentin

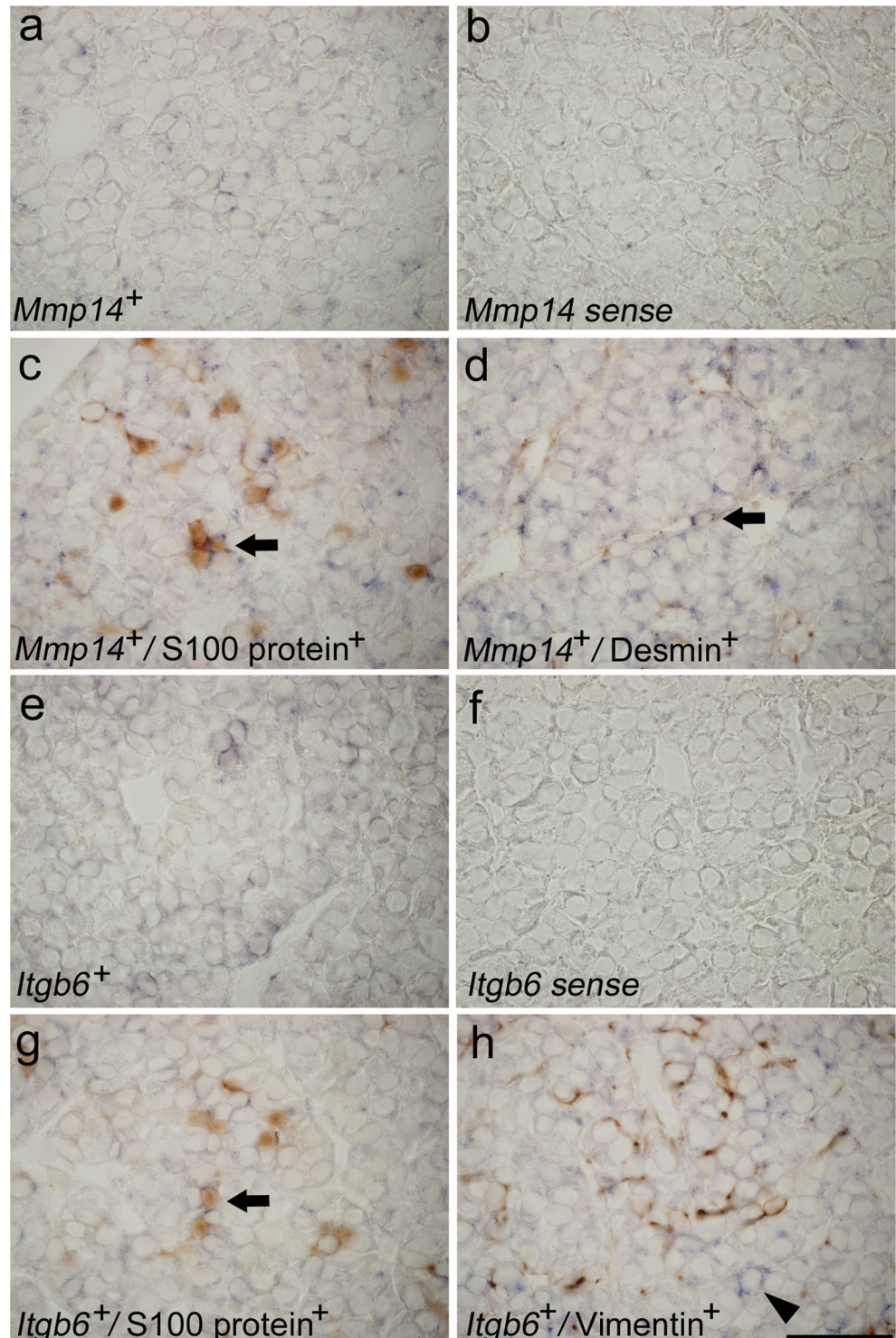
(**b**, white) and GFAP (**c**, red). **d** Merged image of **a-c** (arrowhead a cell immunonegative for vimentin and GFAP). Bar 10  $\mu$ m

indicating that S100 $\beta$ -positive cells are easily separated into two types by making use of their different adherence properties.

To characterize the round and process type S100 $\beta$ -positive cells, we analyzed their mRNA levels by real-time PCR (Fig. 2). *Vimentin* (*Vim*), *Gfap*, *caveolin 3* (*Cav3*), *biglycan* (*Bgn*), *CD11b* (*Cd11b*), and *Il6* have been shown to be

expressed in S100 $\beta$ -positive cells (Allaerts and Vankelecom 2005; Horiguchi et al. 2011, 2013). Among them, expression levels of *Vim*, *Gfap* (for intermediate filament proteins), *Cav3* and *Bgn* (for ECM protein) were higher in the process type than in the round type cells (Fig. 2a-d). In contrast, the round type prominently expressed *Cd11b* and *Il6*, which are markers of dendritic cells (Springer et al. 1979; Fleming et al. 1993;

**Fig. 4** Characterization of *Mmp14*- or *Itgb6*-expressing cells in anterior lobe. mRNA and protein were shown in blue (NBT/BCIP) and brown (3,3'-diaminobenzidine), respectively. In situ hybridization for *Mmp14* with antisense probe (a) and sense probe (b) followed by immunohistochemistry for S100 protein (c) and desmin (d). In situ hybridization for *Itgb6* with antisense probe (e) and sense probe (f) followed by immunohistochemistry for S100 protein (g) and vimentin (h). The arrow indicates a cell double-positive for in situ hybridization and immunohistochemistry. The arrowhead indicates a cell positive for in situ hybridization but negative for immunohistochemistry. Bar 50  $\mu$ m





Allaerts and Vankelecom 2005; Fig. 2e, f). These findings suggest that the process type cells should be categorized as astrocyte- or epithelial-like classic S100 $\beta$ -positive cells, whereas the round type cells resemble dendritic cells. However, the round type might also be a precursor cell of the process type or vice versa.

We recently observed an invasion of mesenchymal cells into the anterior lobe (Higuchi et al. 2013; Yako et al. 2013) suggesting that extra-pituitary cells invade and participate in vasculogenesis in the developing anterior lobe of fetal pituitary. The origin of the round type is an interesting issue, since the dendritic cell might have a non-pituitary origin. Immunohistochemistry with antibodies for several vascular markers has shown that several types of vascular cells are present, indicating that invading mesenchymal cells might differentiate during their invasion. Similarly, we reported an interesting characteristic of the pituitary cell line, TtT/GF, which expresses S100 protein and has been used as a model cell of the folliculo-stellate cell. This cell line does not show properties of an endocrine cell but remarkably exhibits stemness with the expression of the drug-efflux transporter, ABCA1 (Chapman et al. 2003), ABCG2 and other genes (Mitsuishi et al. 2013).

In the present study, we further analyzed the gene expression correlated with ECM signaling to characterize the difference between the process and round type. We found that the process type expressed *Mmp14* and that the round type expressed *Itgb6* (Fig. 2g, h). Commonly, cells utilize MMPs to degrade ECM for the promotion of migration in the tissue (Itoh 2006). MMP14 degrades a number of ECM, such as collagen, laminin, fibronectin and vitronectin (d'Ortho et al. 1997; Ohuchi et al. 1997; Büttner et al. 1998; Fosang et al. 1998). We revealed, in previous reports, that a number of S100 $\beta$ -positive cells in primary culture migrate on laminin-coated surfaces (Horiguchi et al. 2010, 2011). These findings suggest that the process type also expresses *Mmp14* to degrade laminin and migrate. On the other hand, integrins are cell-adhesion receptors that mediate cell-ECM interactions and *Itgb6* is known to be a receptor for fibronectin but not for vitronectin, laminin, or collagen (Breuss et al. 1993, 1995). Fibronectin is also expressed in S100 $\beta$ -positive cells in the rat anterior pituitary (Liu et al. 1989). Thus, we suggest that fibronectin is an autocrine and/or a paracrine factor in the anterior pituitary and that integrin  $\beta$ 6 functions as a fibronectin mediator.

To observe the process type S100 $\beta$ -positive cells in vivo, we examined the localization of vimentin and GFAP by immunohistochemistry in the S100 $\beta$ -GFP rat anterior lobe (Fig. 3a-d). Vimentin-immunoreactive (Fig. 3b) and GFAP-immunoreactive (Fig. 3c) signals were detected in a number of S100 $\beta$ -positive cells (Fig. 3d) throughout the anterior lobe. However, a small number of S100 $\beta$ -positive cells were immunonegative for both proteins (Fig. 3a, e, arrowhead).

We suggest that S100 $\beta$ -positive cells negative for vimentin and GFAP are of the round type. *Mmp14* and *Itgb6* mRNAs were detected in the anterior lobe by in situ hybridization with a DIG-labeled antisense cRNA probe (Fig. 4a-h). A specific signal was not detected in the sections processed with the DIG-labeled sense cRNA probe of *Mmp14* or *Itgb6* (Fig. 4b, f). We could not detect *Cd11b* and *Il6* (inflammatory cytokine) by in situ hybridization in vivo (data not shown). We suggest that their expression levels are extremely low in normal anterior lobe. Instead, we used *Itgb6* as a marker for dendritic-cell-like S100 $\beta$ -positive cells in the present study. We performed double-staining by using in situ hybridization for *Mmp14* and *Itgb6* mRNAs and immunohistochemistry to detect pituitary hormones, S100 protein and desmin. *Mmp14* mRNA was detected in S100-positive cells and desmin-positive cells (Fig. 4c, d, arrows). However, it was not expressed in ACTH-, GH-, PRL-, TSH- and LH-producing cells (data not shown). In addition, *Itgb6* mRNA was only detected in S100-positive cells (Fig. 4g, arrow). However, it was not expressed in vimentin-positive cells (Fig. 4h, arrowhead). These data indicate that cells expressing *Itgb6* are of the round type of S100 $\beta$ -positive cells and that *Itgb6* is a good tool for detecting the round type in vivo.

In conclusion, we developed a simple and prompt method for separating the subpopulation (dendritic-cell-like type and others) of S100 $\beta$ -positive cells from the rat anterior lobe. Although further studies are needed to determine the function and origin of S100 $\beta$ -positive cells, this method allows us to characterize these cells, particularly the dendritic-cell-like S100 $\beta$ -positive cells.

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