Diet-Induced Obesity in Stem Cell Antigen-1 KO Mice

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Stem Cell Antigen-1 (Sca-1) is a member of the lymphocyte-activated protein 6 family and has served as a marker for the identification of stem cells in various tissues, including fat depots. *In vitro* and *in vivo* studies suggest the possible involvement of Sca-1 in adipogenic differentiation and link Sca-1 antigenicity with adipocyte progenitors. Previously, we showed that Sca-1-enriched populations of ear mesenchymal stem cells possess enhanced capacity to differentiate into adipocytes. Additionally, we determined the natural frequency and localization of Sca-1-positive progenitor/stem cells in brown and white fat *in situ*. The present study addressed the question whether Sca-1 deficiency alters the white adipose tissue response to a high-saturated-fat diet. Our results show that Sca-1 null mice (Sca-1^{-/-}) fed high-fat diet developed obesity equally well as wild-type mice, suggesting either an indirect *in vivo* effect of Sca-1 or a compensatory response to Sca-1 deficiency. However, contrary to wild-type mice, high fat diet-fed Sca-1^{-/-} mice showed no alterations in serum adipocytokines. The data lead to the conclusion that Sca-1 is either redundant or a nonessential marker of adipose progenitor/stem cells. Nevertheless, since Sca-1-deficient mice displayed elevated blood glucose at fasting and exhibited glucose intolerance and insulin resistance, Sca-1 has subtle effects on adipose function. Thus, the Sca-1-deficient mice may provide a useful model for metabolic studies.

Introduction

STEM CELL ANTIGEN-1 (Sca-1), a surface protein initially identified as an antigenic marker of murine hematopoietic cells, is commonly used for the identification of stem cells in various tissues, including adipose-derived stem cells (ASC) [1–4]. Several *in vitro* and *in vivo* studies using both Sca-1-enriched/depleted cell fractions (Sca-1⁺/Sca-1⁻) and Sca-1 null mice (Sca-1^{-/-}) have been undertaken to understand Sca-1 functions and mechanisms by which the protein influences cell fate. Many of those studies support the involvement of Sca-1 in adipogenic differentiation. For example, Sca-1⁺ calvarial cells expressed at higher levels of peroxisome proliferator activated receptor γ (PPAR γ), an adipogenic transcription factor, relative to Sca-1⁻ cells [5]. Likewise, Sca-1⁺ endothelial cell and myogenic progenitors identified in the interstitial spaces of murine skeletal muscle display the potential to differentiate into adipocytes [6]. Further, colony-forming unit adipocyte assays have determined that Sca-1^{-/-} bone marrow-derived cells formed 50% fewer colonies compared with wild-type (Sca-1^{+/+}) cultures [7]. Moreover, Sca-1-enriched population of cells identified in neonatal mouse skin expressed adipocyte markers [8]. More recent studies have associated Sca-1 antigenicity with adipocyte progenitors in fat depots [9], and marrow-derived cells

from the Sca-1^{+/+} mice have exhibited a significantly larger number of Oil Red O-stained colonies [10]. In addition, within the adipose tissue stroma, an Sca-1-positive subpopulation of early adipocyte progenitor cells has been identified in murine adipose depots capable of proliferating and differentiating into an adipose depot *in vivo* [11]. These newly differentiated mature adipocytes were also able to reconstitute a fully functional adipose depot *in vivo* [11]. Consistently with this body of evidence, our previous report showed that Sca-1-enriched population of ear mesenchymal stem cells (EMSC) possessed enhanced capacity to differentiate into adipocytes [12]. Additionally, we have determined the natural frequency and localization of progenitor/stem cells in brown and white adipose depots *in situ* based on their ability to retain DNA nucleotide label (5-bromo-2'-deoxyuridine); the majority of these cells were also positive for Sca-1 [3].

Despite this growing body of evidence identifying Sca-1 as a marker for adipose stem or progenitor cells, the fact remains that Sca-1^{-/-} mice do not display lipodystrophy or the absence of some or all adipose depots *in vivo*. The Sca-1^{-/-} mice continue to develop apparently normal subcutaneous and visceral adipose tissues during development *in utero* [13]. Adipose tissue expansion continues after birth through both cell proliferation (hyperplasia) and growth (hypertrophy) of individual adipocytes, ultimately leading to obesity

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[14,15]. This can be induced experimentally by placing mice on a high-fat diet. It is possible that the Sca-1^{-/-} phenotype exerts more subtle influences on adipose depots that are only apparent in the growing adolescent or adult animal. To explore this question, the present study subjected Sca-1^{-/-} versus wild-type mice to a high-saturated-fat diet and tested whether Sca-1 deficiency alters the white adipose tissue response to an obesogenic stimulus. The results suggest that Sca-1^{-/-} phenotype modulates some, but not all, aspects of the adipose tissue response and that Sca-1 is a redundant or nonessential protein with respect to adipogenesis *per se*.

Materials and Methods

Animals

The C57BL/6J (wild type) mice used in the study were obtained from The Jackson Laboratory (Bar Harbor, ME). Sca-1 null mice (Sca-1^{-/-}), generated from C57BL/6J, were characterized and kindly provided by William L. Stanford [13]. Both strains were bred at the Pennington Biomedical Research Center. Mice were housed in a temperature- and humidity-controlled room (22±2°C and 30%–70%, respectively) with a 12-h-light/12-h-dark cycle (lights on at 0600 h) and were allowed *ad libitum* access to food and tap water throughout the study. After weaning, mice were fed a standard low-fat chow diet (LabDiet 5053; PMI, Brentwood, MO) until 8 weeks of age. Then, C57BL/6J and Sca-1^{-/-} mice were randomized into experimental groups, and fed either standard low-fat chow diet or a high-saturated-fat diet (58 kcal% fat w/sucrose; D12331; Research Diets, New Brunswick, NJ). These diets were maintained throughout the experiment. Every 3 weeks, body composition was analyzed by nuclear magnetic resonance (NMR; Bruker, The Woodlands, TX). At the age of 30 weeks the mice were euthanized by CO₂ asphyxiation followed by cervical dislocation. All experiments involving animals were reviewed and approved by the Pennington Biomedical Research Center Institutional Animal Care and Use Committee before implementation in accordance with NIH guidelines. All procedures were designed to minimize the suffering of experimental animals.

RNA isolation and quantitative real-time reverse transcriptase–polymerase chain reaction

Total RNA was extracted using Trizol (Invitrogen, Carlsbad, CA). RNA was further purified by using RNeasy and RNase-Free DNase kits (Qiagen, Valencia, CA). The quantity and quality of RNA were determined by spectrophotometer at 260/280 nm (NanoDrop Technologies, Wilmington, DE). cDNA synthesis was performed with 500 ng of total RNA using the High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA). Endogenous mRNA levels for adiponectin, leptin, and resistin were measured with Applied Biosystems Taqman[®] Gene Expression Assays (Applied Biosystems). Reactions were performed in MicroAmp Optic 384-well Reaction Plates (Applied Biosystems) using the ABI Prism 7900 Sequence Detection System (Perkin Elmer, Boston, MA). The quantitative real-time polymerase chain reaction was performed in duplicates, and each run included a standard curve, nontemplate control, and negative reverse transcriptase (RT) control. All the gene expression data were normalized to the level of hypoxanthine phosphoribosyl-

transferase 1 chosen as the internal control gene based on published data from our own [12] and others [16].

Metabolite and hormone determinations

Blood samples were collected by cardiac puncture and allowed to clot. Samples were then centrifuged at 3,000 rpm for 15 min at +4°C, and the serum was collected and stored at –20°C until assayed. The multiplex assay was performed in a 96-well filter-bottom plate to determine adiponectin, leptin, and resistin concentrations in blood serum according to manufacturer's protocol (Millipore Corporation, Billerica, MA). Briefly, the multibiomarkers and cytokines standards were resuspended in assay buffer and then serially diluted. Twenty-five microliters of standards, quality controls, or the sample was added to each well with 25 µL of the bead solution. The plate was incubated overnight with agitation on a plate shaker at 4°C. Then, the plate was washed twice followed by addition of 25 µL of a detection antibody cocktail. The samples were incubated at room temperature for 1.5 h followed by the addition of streptavidin–phycoerythrin solution and incubation at room temperature for 30 min. The plate was then read using a Luminex reader (Luminex Inc, Austin TX). Glucose tolerance and insulin tolerance tests were performed after overnight fasting using an intraperitoneal injection of 20% glucose solution (2 g/kg of body weight) or insulin (0.5 IU/kg of body weight; Humulin R, Eli Lilly and Company, Indianapolis, IN). Blood glucose levels were determined using an OneTouch Profile blood glucose meter (LifeScan Inc., Milpitas, CA).

Flow cytometry assay

Additional groups of C57BL/6J and Sca-1^{-/-} mice were used to characterize the immunophenotype of stromal-vascular fraction (SVF) cells isolated from white adipose fat depots. The mice were maintained on standard chow diet throughout the whole study period. At the age of 30 weeks epididymal and inguinal fat depots were collected, minced, and digested with collagenase type I (2 mg/mL; Worthington Biochemical Corp., Freehold, NJ). Red blood cell lysis buffer (Sigma Co., St. Louis, MO) was used to remove erythrocyte contamination before analyzing by flow cytometry. Cells isolated from the fat depots were incubated with phycoerythrin-conjugated antiSca-1, antiCD44, antiCD45, antiCD73, antiCD90.1, antiCD-90.2, and antiCD117 antibodies (BD Pharmingen, San Diego, CA), as previously described [17]. The flow cytometry assay was performed using a FACScan flow cytometer (Becton Dickinson, San Jose, CA) and data were analyzed with a Macintosh G5 workstation (Apple Computer, Cupertino, CA), which contains Cellquest graphics software (Becton Dickinson) for data acquisition and analysis. Flow cytometry (FACS) plots and data are representative of 3 separate experiments.

Histological analysis of fat depots

Formalin-fixed inguinal and epididymal fat depots were embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin. Slides were viewed at magnification (×10) using a Zeiss microscope (Axioskop 40) using Plan-291 Neofluor objective and photographed with a Kodak digital camera (DC290 Zoom). Quantitative analyses of adipocytes

were made using Metamorph software. Cells were counted from 5 randomly chosen fields.

In vitro differentiation assays

Primary cultures of Sca-1⁺ and Sca-1⁻ASC were isolated from the inguinal fat pads of C57BL/6J mice using magnetic beads as previously published [12]. The established adherent cultures were induced to undergo adipogenic differentiation by addition of DMEM/F12, 5% FBS, 1% antibiotic solution, 0.5 mM isobutylmethylxanthine, 1.7 μ M insulin, and 1 μ M dexamethasone for 2 days, followed by 6-day exposure to DMEM/F12 medium supplemented with 5% FBS, 1% antibiotic solution, 17 nM insulin, and 2 μ M thiazolidinedione [12]. After 8 days in culture, representative cultures were fixed and stained for lipid accumulation with Oil Red O or harvested for total RNA used in real-time polymerase chain reaction analysis of the adipocyte-associated leptin and PPAR γ 2 according to published methods [12].

Statistical analysis

Data were analyzed with Prism 5 (GraphPad Software, Inc., San Diego, CA) and expressed as mean $\pm$ standard deviation. Student's *t*-test was used to compare between 2 groups. A value of *P* < 0.05 was considered statistically significant.

Results

Diet

Mice introduced to both chow and high fat adapted immediately to the diets. Over the course of 30 weeks there was no difference in increase of body weight between the strains placed on either respective diet (Fig. 1A). As expected, mice fed high-fat diet were heavier than mice fed chow diet.

Body composition

Every 3 weeks, NMR was performed to assess body composition. Consistent with the similarity in body weight, NMR showed the expected increase in percentage body fat for mice fed high-fat diet; there was no difference observed between the strains (Fig. 1B). Regardless of both the diet and strain, all mice demonstrated the same patterns of lean mass (Fig. 1C).

Hormonal and metabolic profiles

In wild-type mice the different diets were associated with significant changes in blood serum concentrations of adiponectin (decrease), and of leptin and resistin (increase) (Fig. 2B, D, and F, respectively) in high fat diet-fed animals relative to chow-fed controls. In Sca-1^{-/-} mice, only resistin levels were influenced by diet, being higher in high-fat diet fed (Fig. 2F). Adiponectin concentration in blood serum was found to be elevated in Sca-1^{-/-} mice versus wild-type mice placed on high-fat diet (Fig. 2B). Significant differences between the analyzed strains were additionally observed in resistin serum concentration; Sca-1^{-/-} mice showed increased resistin in chow-fed animals compared with C57BL/6J controls (Fig. 2F). At the mRNA level, the inguinal fat depot isolated from chow diet-fed mice showed higher ex-

FIG. 1. High-fat (HF) diet leads to adiposity: (A) body weight, (B) fat, and (C) lean mass trajectories (chow: *n* = 8; HF: *n* = 8). Data are mean $\pm$ SD. SD, standard deviation.

pression levels of adiponectin and resistin mRNAs in wild-type mice (Fig. 2A and E, respectively) compared with high-fat diet fed.

Glucose and insulin sensitivity

Glucose tolerance (Fig. 3A and B) and insulin sensitivity (Fig. 3C and D) tests were performed with wild-type and Sca-1^{-/-} mice. Regardless of the diet, basal fasting glucose was lower in wild-type mice compared with Sca-1^{-/-}. After an intraperitoneal glucose tolerance test, the wild-type animals fed a chow diet displayed a much more efficient clearance of systemic glucose levels than Sca-1^{-/-} mice on the same diet (Fig. 3).

Histologic analysis of fat depots

To quantify the number of adipocytes in white fat depots, we counted the number of cells per field in the hematoxylin and eosin-stained section. Consistent with the similarity in

FIG. 2. Effect of diet on mRNA (A, C, E) and protein level (B, D, F) of adiponectin (A, B), leptin (C, D), and resistin (E, F). Data are mean $\pm$ SD. * P < 0.05; ** P < 0.01; *** P < 0.001.

adiposity expressed as body and fat weight, mice fed high-fat diet showed lower number of the cells (Fig. 4). Unexpectedly, the number of cells counted in epididymal fat depot from high fat diet-fed Sca-1^{-/-} mice was lower compared with wild-type mice, consistent with a larger adipocyte volume or hypertrophy in the Sca-1^{-/-} mice.

Adipogenic differentiation of primary ASC

To evaluate the adipogenic differentiation potential of Sca-1⁺ and Sca-1⁻ cell populations, the SVF cells from white fat depots of wild-type C57BL/6J mice were separated by immunomagnetic beads selective for the Sca-1 antigen. The adherent cells were expanded and induced for adipogenesis over an 8-day period (Fig. 5). The Sca-1⁺ population displayed substantially greater adipogenesis relative to the Sca-1⁻ cells (Fig. 5A). Consistent with this, the Sca-1⁻ cells

exhibited significant reductions in PPAR γ 2 (Fig. 5B) and leptin (Fig. 5C) mRNAs relative to equivalent Sca-1⁺ cell cultures and unsorted cells.

Flow cytometry analysis of SVF

C57BL/6J and Sca-1^{-/-} mice SVF cells were phenotyped for immunologically relevant cell surface markers by flow cytometry (Table 1; Figs. 6 and 7). As expected, the analysis demonstrated that wild-type mice highly expressed Sca-1, whereas Sca-1^{-/-} were negative (Fig. 6A and B). Freshly isolated SVF cells from inguinal and epididymal fat depot of Sca-1^{-/-} mice showed slightly higher percentage of cells positive for CD117 than C57BL/6J (Fig. 6C and D). Expression of the stromal-associated surface antigens CD73 (Fig. 7A and B) and CD44 (Fig. 7C and D) in the SVF were present at very low levels (up to ~7.9% and 3.4%,

FIG. 3. Mice fed high-fat (HF) diet demonstrate impaired glucose tolerance and insulin sensitivity to a glucose challenge. **(A)** chow and **(B)** HF blood glucose during a glucose tolerance test (2 g/kg of body weight). **(C)** HF and **(D)** chow insulin sensitivity test (0.5 IU/kg of body weight). Data are mean $\pm$ SD. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, versus wild-type mice.

respectively). Among hematopoietic markers the highest expression levels were found for CD45 (35%–59%; Fig. 6E and F). Moreover, SVF cell populations of C57BL/6J fat depots contained significantly lower percentage of cells expressing CD45 (Table 1) and CD90.1 (Fig. 7E and F) in inguinal and epididymal fat depots, respectively, compared with Sca-1^{-/-} mice.

Discussion

Obesity and weight gain result from an enlarged adipose tissue mass due to an increase in the size of individual and the generation of new adipocytes (hypertrophy and hyperplasia, respectively) [14,15]. The relative role of these processes may be depot specific, as recent high-fat dietary studies indicate that while hypertrophy dominates in visceral fat, hyperplasia is the major contributor to subcutaneous fat expansion [18]. A number of groundbreaking articles have proven the existence of ASC that can be easily isolated from fat depots [19,20]. The cells possess multilineage mesodermal and also possible ectodermal and endodermal potentials that make them a promising tool in the tissue engineering and regenerative medicine. Mesenchymal progenitor cells found in adipose tissue have been considered as a potential source of newly formed lineage-committed preadipocytes/adipocytes, although a controversy exists between in vitro and in vivo studies in this field [21–23]. In the present study, the flow cytometry analysis confirmed that SVFs isolated from both epididymal and inguinal white adipose depots include a large population of mesenchymal stem cells. The cells expressed the stromal-associated surface antigens (CD44, CD73), and stem cell marker (Sca-1; wild-type mice only). This is in agreement with previously published reports [1]. Consistent with other reports, the present study demonstrates the additional presence of hematopoietic

cell markers in the SVF [24,25] as well as an initially low population of cells expressing stromal cell-associated markers [4,26].

Despite the wealth of information relating to the cellular and molecular mechanisms that underlie adipocytes/preadipocytes generation, the complexity of adipose tissue development continues to present an active area of investigation. In the present study we employed mice deficient in the cell surface protein Sca-1 to test the in vitro-based hypothesis that Sca-1 promotes adiposity.

The high-saturated-fat and control chow diets were maintained throughout the experiment. When mice were fed a high-fat diet, the outcome was not surprising; the animals' body weight, body fat, and fat cell size increased, and reduced skeletal muscle mass relative to body weight was observed. However, unexpectedly Sca-1 depletion did not alter adiposity or lean mass in Sca-1^{-/-} mice fed high-fat diet. This observation was opposite to the significant number of in vitro studies that showed enhanced ability of adipogenic differentiation in Sca-1-expressing cells [5–8,10–12]. Moreover, in vivo studies by Crossno et al. showed that exposure to high-fat diet promoted a recruitment of circulating bone marrow-derived progenitor cells into adipose tissue, and their differentiation into adipocytes; interestingly, those cells also expressed Sca-1 [9].

Adipose tissue is a primary site for energy storage. It is also recognized as an endocrine organ capable of secreting hormones and cytokines, which are essential determinants of energy intake and expenditure *via* their autocrine, paracrine, and endocrine actions. Adipocytokines such as adiponectin, leptin, and resistin play a key role in energy homeostasis and insulin resistance [27–29]. As expected, in the present study, obese, high fat diet-fed wild-type mice showed higher leptin [30–32] and lower adiponectin [33] blood serum concentration; however, comparably fed Sca-1^{-/-} mice showed no

FIG. 4. Quantitative analysis of adipocytes in hematoxylin and eosin-stained slides of white fat depots from C57BL/6J and Sca-1^{-/-} mice. Data are mean $\pm$ SD. * $P < 0.05$ versus wild-type mice.

alterations in serum adipocytokines. The elevated adiponectin level goes hand in hand with low leptin level and that inverse relationship between those 2 circulating adipocytokines has been reported previously [34]. In our study resistin was the only analyzed peptide that responded to high-fat diet with elevated levels, regardless of the strain. Increased circulating level of this hormone is a common feature of obese rodents [35].

The majority of leptin is derived from white adipose tissue [36–38], and its mRNA and plasma levels correlate linearly with mass of adipose tissue and fat cell size [32,39,40]. In our study the higher rates of leptin biosynthesis in obese mice tightly correlated with leptin mRNA. Leptin gene expression levels in fat depots were elevated in high fat in comparison to chow diet-fed mice. Further, in epididymal fat depots collected from high fat diet-fed Sca-1^{-/-} mice the leptin mRNA level was increased. In contrary, adiponectin and resistin mRNA expression levels were elevated only in inguinal fat depots of wild-type chow diet-fed mice. These molecular

findings are not consistent with the previously reported difference in leptin expression displayed between the Sca-1-enriched and Sca-1-depleted ear mesenchymal stem cells fractions during in vitro adipogenic differentiation [12]. Whereas the Sca-1⁺ fraction showed substantial increase in leptin mRNA, the Sca-1-depleted fraction expressed leptin at low levels. Similarly, our present in vitro study showed high levels of leptin mRNA in Sca-1-positive cells and low levels in Sca-1-negative ASC.

Sca-1, also known as lymphocyte-activated protein 6 a (Ly6a), is a surface anchored glycosyl phosphoinositol (GPI) protein [41]. The Ly6 family includes 18 members, many of which are associated with lipid rafts and have the potential to act in cell adhesion/receptor processes [41]. The human homolog for Sca-1 has not yet been definitively identified [41]. Sca-1 has been implicated as a stem cell biomarker in multiple tissue sites, including bone marrow, cardiovascular, hematopoietic, liver, lung, mammary gland, prostate, and other tissues [41]. For example, Sca-1 has been found on

FIG. 5. Robust adipogenic differentiation of Sca-1⁺ cells. **(A)** Phase-contrast micrographs of Sca-1⁺ and Sca-1⁻ population of magnetically separated SVF cells from iWAT of C57BL/6J mice at day 8 of adipogenic differentiation. **(B, C)** Quantitative real-time polymerase chain reaction analysis of PPAR γ 2 **(B)** and leptin **(C)** expression in cultured unsorted, Sca-1⁺ and Sca-1⁻ population of SVF at days 0 and 8 of adipogenic differentiation. Data are mean $\pm$ SD; ** P < 0.01; *** P < 0.001. Sca-1, stem cell antigen-1; SVF, stromal-vascular fraction; PPAR γ 2, peroxisome proliferator activated receptor γ 2.

TABLE 1. IMMUNOPHENOTYPIC CHARACTERIZATION OF MOUSE STROMAL-VASCULAR FRACTION CELLS ISOLATED FROM WHITE ADIPOSE FAT DEPOTS FROM C57BL/6J AND SCA-1^{-/-} MICE BY FLOW CYTOMETRIC ANALYSIS

	<i>Epididymal</i>		<i>Inguinal</i>	
	<i>Bl6</i>	<i>Sca-1KO</i>	<i>Bl6</i>	<i>Sca-1KO</i>
Sca-1	44.8 $\pm$ 1.5	nd	55.8 $\pm$ 4.5	nd
CD45	55.4 $\pm$ 1.6	59.1 $\pm$ 2.3	35.6 $\pm$ 6.3 ^a	50.2 $\pm$ 4.6
Sca-1/CD45	11.9 $\pm$ 1.7	nd	9.4 $\pm$ 1.1	nd
CD117	14.1 $\pm$ 3.3	19.1 $\pm$ 1.2	7.4 $\pm$ 0.5	10.2 $\pm$ 3.7
Sca-1/CD117	3.7 $\pm$ 1.4	nd	2.4 $\pm$ 0.3	nd
CD73	7.1 $\pm$ 1.3	7.9 $\pm$ 0.6	3.6 $\pm$ 1.5	5.2 $\pm$ 0.2
CD44	2.6 $\pm$ 0.8	3.0 $\pm$ 0.2	1.7 $\pm$ 0.3	3.4 $\pm$ 1.9
CD90.1	2.3 $\pm$ 0.2 ^b	3.5 $\pm$ 0.1	3.1 $\pm$ 0.7	3.6 $\pm$ 1.5
CD90.2	26.9 $\pm$ 1.8	20.0 $\pm$ 4.7	27.1 $\pm$ 2.3	31.9 $\pm$ 2.4

^a P < 0.05; ^b P < 0.001.

nd, nondetected.

skeletal muscle progenitor cells, where it is associated with insulin-degrading enzyme [42]. Since insulin-degrading enzyme catabolizes mitogenic peptides, this may underlie the signaling pathway mediating Sca-1 actions [42]. Likewise, the function of Sca-1 has been evaluated extensively in lymphoid cell models where downstream kinase mechanisms have been implicated but not proven [41]. Further work will be required to address the mechanistic role of Sca-1 at the biochemical level in adipose stromal/stem cells.

At present, our results suggest that Sca-1 may not contribute directly to adipose tissue characteristics associated with obesity. We have shown that Sca-1-deficient mice developed obesity equally well as wild-type mice, and Sca-1^{-/-} had no significant effect on adiposity in mice. This observation could be due to an indirect in vivo effect of Sca-1 or may suggest a compensatory response to Sca-1 knock-down. Our work leads to the conclusion that Sca-1 is either redundant or a nonessential marker of adipose stromal/stem cells. It is feasible that another related member of the Ly6 family can substitute for Sca-1 and allow Sca-1^{-/-} adipose stromal/stem cells to retain some, if not all, functionality.

FIG. 6. A representative flow cytometric analysis of freshly isolated SVF of epididymal fat depot from C57BL/6J and Sca-1^{-/-} mice. Cells were labeled with phycoerythrin (Sca-1) PerCP (CD45), and fluorescein isothiocyanate (CD117) antibodies. The percentage of cells staining positive is indicated on each panel. The data are representative of 3 experiments; each experiment consisted of a pool of cells collected from 3 animals.

FIG. 7. A representative flow cytometric analysis of freshly isolated S-V fraction of epididymal fat depot from C57BL/6J and Sca-1^{-/-} mice. Cells were labeled with phycoerythrine (CD73, CD44, CD90.1, and CD90.2) antibodies. The percentage of cells staining positive is indicated on each panel. The data are representative of 3 experiments; each experiment consisted of a pool of cells collected from 3 animals.

Nevertheless, since Sca-1^{-/-} mice display elevated blood glucose at fasting and exhibited glucose intolerance and resistance to the hormone insulin, there is evidence that Sca-1 deficiency has subtle effects on adipose function. Consequently, the Sca-1^{-/-} mice may provide a useful model for future studies of metabolic disorders.

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Author Disclosure Statement

The authors declare no competing financial interests.

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