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Original Research

Telomerase Reverse Transcriptase Expression Marks a Population of Rare Adipose Tissue Stem Cells

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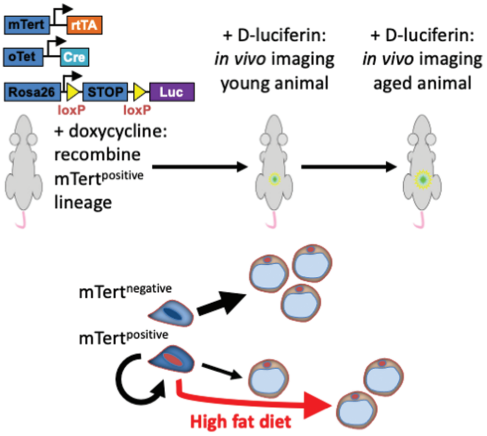
Abstract

In adult tissues such as adipose tissue, post-mitotic cells like adipocytes can be replaced by differentiation of a population of tissue-resident stem cells. Expression of mouse telomerase reverse transcriptase (mTert) is a hallmark of stem cell populations, and previous efforts to identify tissue-resident adult stem cells by measuring mTert expression have increased our understanding of stem cell biology significantly. Here, we used a doxycycline-inducible mouse model to perform longitudinal, live-animal lineage-tracing of mTert-expressing cells for more than 1 year. We identified a rare (<2%) population of stem cells in different fat depots that express putative preadipocyte markers. The adipose-derived mTert-positive cells are capable of self-renewal and possess adipogenic potential. Finally, we demonstrate that high-fat diet (HFD) can initiate differentiation of these cells in vivo. These data identify a population of adipose stem cells that contribute to the depot-specific response to HFD.

Key words: adipose tissue; telomerase; adipogenesis; adult stem cells.

Graphical Abstract



Mouse telomerase reverse transcriptase (mTert) is expressed in adult tissue stem cell populations. To trace the lineage of adults cells that express mTert, an inducible lineage tracing mouse model was followed longitudinally using bioluminescent imaging to identify a population of adipocyte precursor cells that reside in the stromal vascular fraction, where they are capable of self-renewal and adipogenic differentiation.

Significance Statement

Stem cells express high levels of telomerase reverse transcriptase to maintain telomere length. We followed the lineage of these telomerase-expressing cells in adult transgenic animals using lineage tracing. We traced telomerase-positive cells longitudinally in mice for almost 2 years and found that a population of these cells reside in adipose tissue, where they form a subset of putative mesenchymal adipocyte progenitor cells and respond to high-fat diet by differentiating into mature adipocytes. These results identify a rare population of adipose tissue adult stem cells which may be key to the response to high-fat diet in fat tissue.

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Introduction

Stem cells are characterized by a long lifespan, which is required to act as a reservoir to replace terminally differentiated, post-mitotic cells over time.[1] Cellular aging and the so-called "mitotic clock" are controlled in part by the shortening of telomeres at the ends of chromosomes during each round of mitosis.[2] To maintain telomeres and extend their lifespan, stem cells express telomerase reverse transcriptase (Tert), the catalytic subunit of telomerase, the ribonucleoprotein complex responsible for catalyzing the extension of telomeric DNA.[3-5] In mice, mTert expression is tightly correlated with telomerase activity and exhibits a strong negative correlation with differentiation state.[5, 6] Concordant with this, Tert is expressed in many self-renewing tissues and undifferentiated cell types such as ES cells[6] and its expression pattern has been used as a proxy for self-renewal to identify adult tissue-resident stem cells in bone marrow [7] and within the intestinal crypt.[8] Therefore, mTert expression is a useful biomarker for identifying and functionally studying stem cells in adult tissues such as adipose.

Adipocytes must be continuously replaced during life from a pool of progenitor cells,[9] and adipose progenitor cells with high genomic stability been identified in mice and humans.[10,11] The adipocyte cellular lineage has been characterized using a combination of lineage-tracing and cell sorting strategies, which has led to the identification of cell surface antigens that mark a population of committed precursor cells in adipose lineage such as platelet-derived growth factor receptors α and β (PDGFrα/β), CD34, CD29, CD24, and stem cell antigen 1 (Sca1).[12-16] In humans, adipose-mesenchymal stromal cells stain positive for CD44, CD73, CD90, CD105, CD34, and CD13 while staining negative for markers: CD31, CD45, and CD235a.[17-20] Indeed PDGFrα/β gene expression has been used in lineage-tracing studies to label adipocytes precursor cells in virtually all adipose depots. In addition to these common adipose precursor markers, brown adipocytes arise from precursors that are $Myf5^{positive}$ while white and beige adipocyte precursors, for the most part, arise from a cellular lineage that is $Myf5^{negative}$.[21-23] Recently, single-cell RNA sequencing has allowed for the identification of new markers for novel populations of adipose precursor cells.[24-28] For example, 2 populations of PDGFr-positive cells have now been described, the $PDGFr\alpha^{pos}$ adipose progenitors and the $PDGFr\beta^{pos}Ly6c^{pos}$ fibro-inflammatory progenitors in gonadal white adipose tissue (WAT).[29,30] Interestingly, Tert expression in these putative progenitor populations can regulate senescence, and transgenic animals lacking Tert display functional impairments in both subcutaneous white adipose tissue (sWAT) as well as whole-body metabolism Tümpel.[31] Despite these important advances, the identity of mTert-expressing tissue-resident adipocyte stem cells (ASCs) and their role in adipogenesis have yet to be elucidated.

Here, we employ lineage-tracing to identify a rare population of mTert-expressing ASCs that are capable of adipogenesis and self-renewal and are responsive to high-fat feeding.

Materials and Methods

Mice

All animal procedures were approved by the Institutional Animal Use and Care Committee at Joslin Diabetes Center. mTert-rtTA mice and mTert-GFP mice were generated in the Breault laboratory.[32] oTet-Cre mice (Stock no. 006224), Rosa26(Luc) mice (Stock no. 005125), and Rosa26(mTom/mGFP) mice (Stock no. 007576) were obtained from the Jackson Laboratory. Mice fed HFD were fed with a diet containing 45% calories from fat obtained from Research Diets (Stock no. D12451). Cold challenged mice were housed at 4°C for 1 week in a diurnal incubator. All mice were allowed ad libitum access to water and food.

Flow Cytometry

Stromal vascular fraction (SVF) was isolated by collagenase digestion and differential centrifugation according to established protocols. Cells were labeled with fluorescent-conjugated antibodies for 20 minutes before they were washed and re-suspended. Antibody dilutions, fluorescent labels, sources, and catalog numbers are given in Supplementary Table 1. Just before analysis, cells were stained with propidium iodide (PI) and analyzed with a BD FACSARIA 2 FACS machine (BD Biosciences). All plots were made using FlowJo software (FlowJo LLC). Cells sorted for co-culture experiments were sorted into microcentrifuge tubes containing Dulbecco's modified Eagle's medium (high glucose) with 10% bovine serum and then plated.

Quantitative RT-PCR

Total RNA was extracted from tissue or cells with Trizol and purified using a spin column kit (Zymo Research). RNA (500 ng-1 µg) was reverse transcribed with a high-capacity complementary DNA (cDNA) reverse transcription kit (Applied Biosystems). Real-time PCR was performed starting with 10 ng of cDNA and forward and reverse oligonucleotide primers (300 nM each) in a final volume of 10 µl with SYBR green PCR Master Mix (Roche). Fluorescence was determined and analyzed in an ABI Prism 7900 sequence detection system (Applied Biosystems). Real-time PCR primer sequences are listed in Supplementary Table 2.

Cell Culture

Adipose tissue was dissected and minced before digestion in 0.2% collagenase I (Worthington). After SVF cells were collected by centrifugation, red blood cells were lysed with NH4Cl and then the SVF was filtered through a 40-µm filter. SVF cells were maintained in Dulbecco's modified Eagle's medium (high glucose) containing 10% bovine serum at 37°C in a 5% CO2 incubator.[12] Adipocyte differentiation was induced in 70% confluent cells by treating cells with an induction mixture containing insulin (10 µg/mL), indomethacin (50 µM), 3-isobutyl-1-methylxanthine (0.5 µM), dexamethasone (1 µM), and triiodothyronine (1 nM). After 48 hours of induction, cells were kept in a medium containing insulin and triiodothyronine for the subsequent 5 days, changing the medium every 3 days. Neurogenic differentiation was induced in 70% confluent cells by treating cells with an induction mixture containing 10 mM 2-mercaptoethanol for 48 hours.[33]

For immunostaining, cells were fixed in formalin, then washed in PBS and permeabilized in 0.25% Triton X-100. After another wash, cells were blocked in 1% BSA, then incubated with primary antibody for 1 hour at room temperature. After another wash, cells were incubated with fluorescent secondary antibody for 20 minutes at room temperature, followed by a final wash and imaging on an inverted microscope. Antibody dilutions, sources, and catalog numbers are given in Supplementary Table 1.

Time Lapse Microscopy

Primary adipose tissue SVF cells were plated on a Hi-Q4 culture dish (Nikon) and cultured in a Nikon BioStation IM-Q which is a compact cell incubator and monitoring system that allows for live-cell imaging. Cells were maintained in BioStation IM-Q at 37°C in a 5% CO2 environment. Bright-field and fluorescent images were obtained every hour for a total of 5 days.

Histology

For tissue histology, freshly isolated adipose tissue was cut into pieces approximately 4 mm^3 and whole mounted onto slides in PBS according to previously published techniques.[34] Whole-mount tissue was imaged using confocal microscopy and images were analyzed using ImageJ software.

In Vivo Bioluminescent Imaging

For acquisition of the bioluminescence images, the mice were sedated with 2% isoflurane. D-Luciferin (Perkin Elmer) was diluted to 3 mg/100 µL in normal saline and 0.6 mg of D-Luciferin was administrated intraperitoneally. All images were acquired 15 minutes after D-Luciferin injection. An IVIS-Spectrum CT imaging system equipped with a CCD camera (Caliper Life Sciences) was used for in vivo bioluminescence imaging. For 3D bioluminiscence, mice were scanned using the IVIS Spectrum CT using the standard single-mouse resolution MicroCT and bioluminescence was projected onto this 3-dimensional data using Living Image software (Caliper Life Sciences).

Replication, Statistical Tests, and Reporting of Sample Sizes

Statistical methods were not used to predetermine the sample size. The experiments were not randomized. All statistics were calculated using Graphpad Prism. The statistical significance was determined by Student's *t*-test. All data needed to evaluate the conclusions in the paper are presented in the paper and/or the Supplementary Materials.

Results

mTert Expression Marks Specific Cellular Lineages In Vivo

To begin to define all of the cells and tissues where mTert is expressed, we consulted the Tabula Muris single-cell gene expression profiling (RNA-Seq) database and noted expression in cells from several tissues including liver, brain, intestine, and adipose tissue.[35] (Supplementary Figs. 1-3). These data suggest different tissues harbor mTert-expressing cells that may act as stem cells.

To identify mTert+ adult stem cells in vivo, we used a mTert lineage-tracing mouse model system (mTert-rtTA::oTet-Cre).[32] In these transgenic animals, expression of the reverse tetracycline controlled transactivator (rtTA) is under the control of the mTert promoter, while expression of the Cre recombinase is controlled by the tet operator (oTet). Feeding these mice doxycycline allows rtTA to bind to the oTet sequence and drive Cre expression, resulting in genetic recombination in cells that express mTert (Supplementary Fig. 4). To visualize stem cells and their descendant cells in live animals we generated trigenic mTert-rtTA-Luc mice by crossing mTert-rtTA::oTet-Cre mice with a Firefly Luciferase reporter line Rosa26(Luc), such that expression of mTert would lead to a permanent genetic marking of all mTert expressing cells and their progeny that can be visualized by measuring Firefly Luciferase activity (Fig. 1A). Next, 10-week-old mTert-rtTA-Luc mice underwent pulse-chase analysis receiving doxycycline for 24 hours followed by in vivo bioluminescent imaging for up to 22 months. To visualize the expression of Firefly Luciferase during the chase, mTert-rtTA-Luc mice received an injection of the Luciferase substrate D-Luciferin starting the day after doxycycline treatment and at 1 week, 2 months, 3 months, 6 months, 10 months, 19 months, and 22 months post-doxycycline treatment, followed by in vivo imaging 15 minutes after D-Luciferin injection (Fig. 1B). Consistent with previous reports for mTert expression, the signal was observed in multiple tissues, including in the chest, legs, pelvic regions, and gonads.[7,8,32,36] By combining luciferase expression with MicroCT imaging, we could project the anatomical location of the lineage-marked cells in 3 dimensions (Fig. 1C,D, Supplementary Video 1). Interestingly, we observed signal in a variety of locations, including brown adipose tissue (BAT) and sWAT. Ex vivo analysis of tissue bioluminescence confirmed signal in adipose tissue as well as in the other tissues previously reported to harbor mTert-positive stem cells, such as liver and small intestine[37,38] (Fig. 1E).

mTert-expressing Cells in Adipose Stromal Vasculature Fraction Express Preadipocyte Markers

Because cells of the mTert lineage have not previously been reported in adipose tissue, we hypothesized that they may act as tissue-resident ASCs. In adipose tissue, mesenchymal preadipocytes are known to reside in the SVF,[39,40] a cellular compartment that can be isolated from mature adipocytes using differential centrifugation. To determine if mTert was expressed in adipose SVF, we first examined the SVF from mTert-GFP mice (Fig. 2A), previously shown to mark progenitor/stem cells in a range of tissues.[41] By excluding hematopoietic lineage (Lin-) cells, we observed GFP expression in approximately 1-3% of the mesenchymal cells in the SVF of white and brown fat (Fig. 2B). Flow cytometric analysis conducted by staining with antibodies specific for putative preadipocyte cell surface markers PDGFrα, PDGFrβ, Sca1, CD29, CD24, and CD34 revealed GFP co-expression in a small subpopulation of cells that expressed these proteins, with almost all GFP+ cells expressing all preadipocyte markers that were interrogated (Fig. 2C, Supplementary Fig. 5). Consistent with previous studies, GFP+ cells expressed high levels of mTert compared to GFP-cells, further validating this mouse model (Fig. 2D). Interestingly, these cells also expressed higher mRNA levels of the putative stem cell marker genes Nanog, Oct4, and Sox2 compared to mTert-GFP- cells (Supplementary Fig. 5E,F). In vitro, mTert-GFP and CD34 were co-expressed in cells after a week of culture (Supplementary Fig. 6) and using time-lapse microscopy, we observed that cultured mTert-GFP+ cells isolated from BAT and sWAT could give rise to daughter cells that maintained mTert-GFP expression for 24 hours or more after mitosis (Fig. 2E).

Taken together, these data indicate that mTert-expressing cells reside in the stromal vasculature of adipose tissue where they make up a subset of the total adipocyte precursor pool.

mTert-expressing Cells Are Capable of Differentiation In Vitro

To determine the adipogenic potential of mTert+ cells, we generated an *in vitro* lineage-tracing system to track the cellular fate of mTert+ cells (Fig. 3A, Supplementary Fig. 7A). For

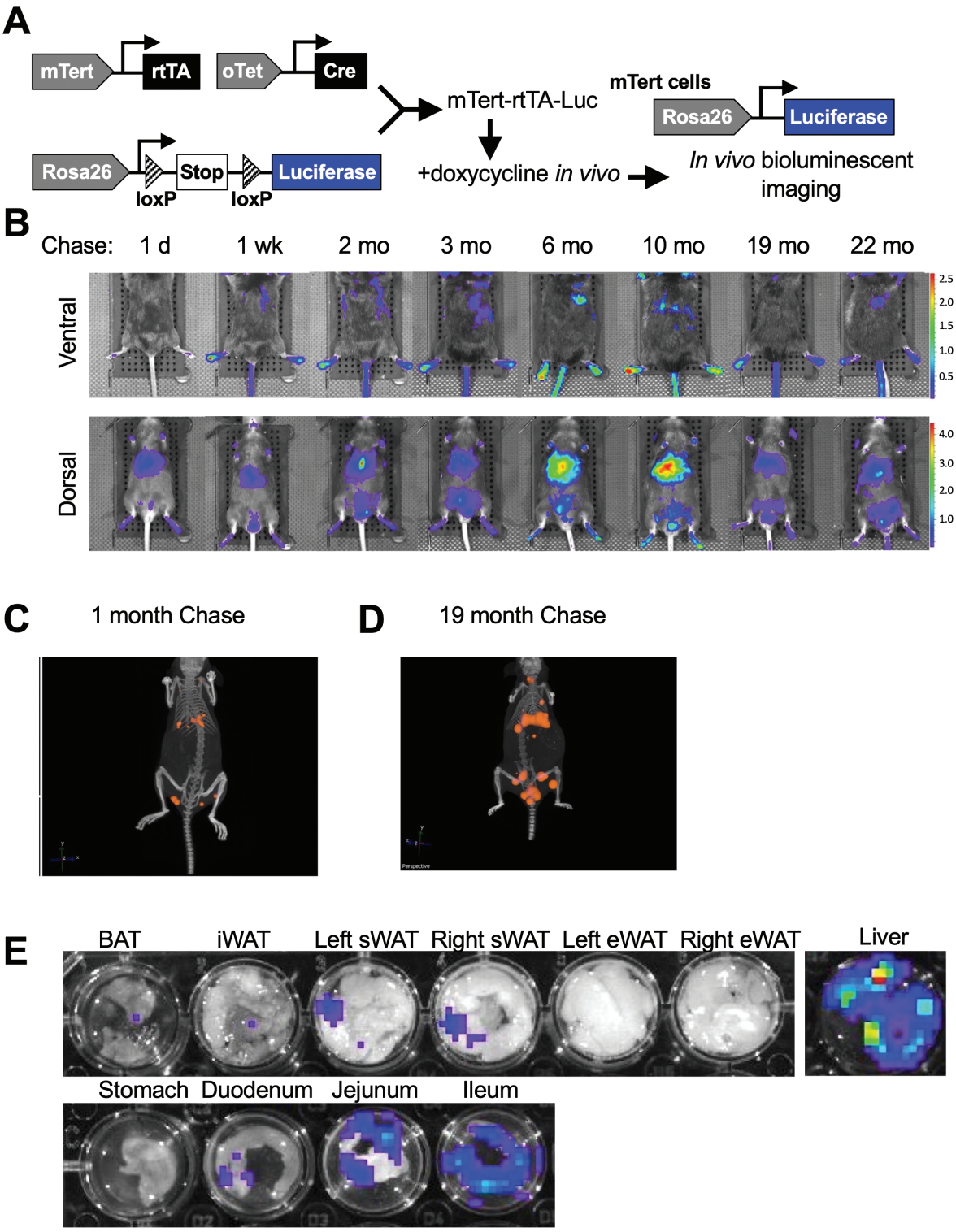

Figure 1. Bioluminescent longitudinal tracing of the mTert lineage in vivo. (**A**) Schematic diagram of lineage-tracing strategy. The cellular lineage of mTert expressing cells was followed using a genetic reporter system wherein doxycycline induces expression of cre recombinase only in cells that express mTert, leading to recombination of mTert expressing cells and constitutive expression of Firefly luciferase, which can be imaged after D-Luciferin injection. (**B**) In vivo bioluminescence of an mTert-rtTA-Luc mouse over 22-month chase from both dorsal and ventral views. mTert+ cells marked during the 1 day pulse of doxycycline given at 10 weeks of age contributed to cells that constitutively express luciferase where ever signal occurs over the life of the animal. (**C,D**) Three-dimensional volume filling model of luminescent signal in mTert-rtTA-Luc mouse after 1 month chase (**C**) and 19-month chase (**D**). Combined luciferase activity data and MicroCT data projecting the location of cells derived from the mTert+ lineage. In each image, an example is shown from *n* = 3-5/group. (**E**) Dissected tissue ex vivo luciferase activity mTert-rtTA-Luc mouse after 19-month chase. In each image, an example is shown from *n* = 3-5/group.

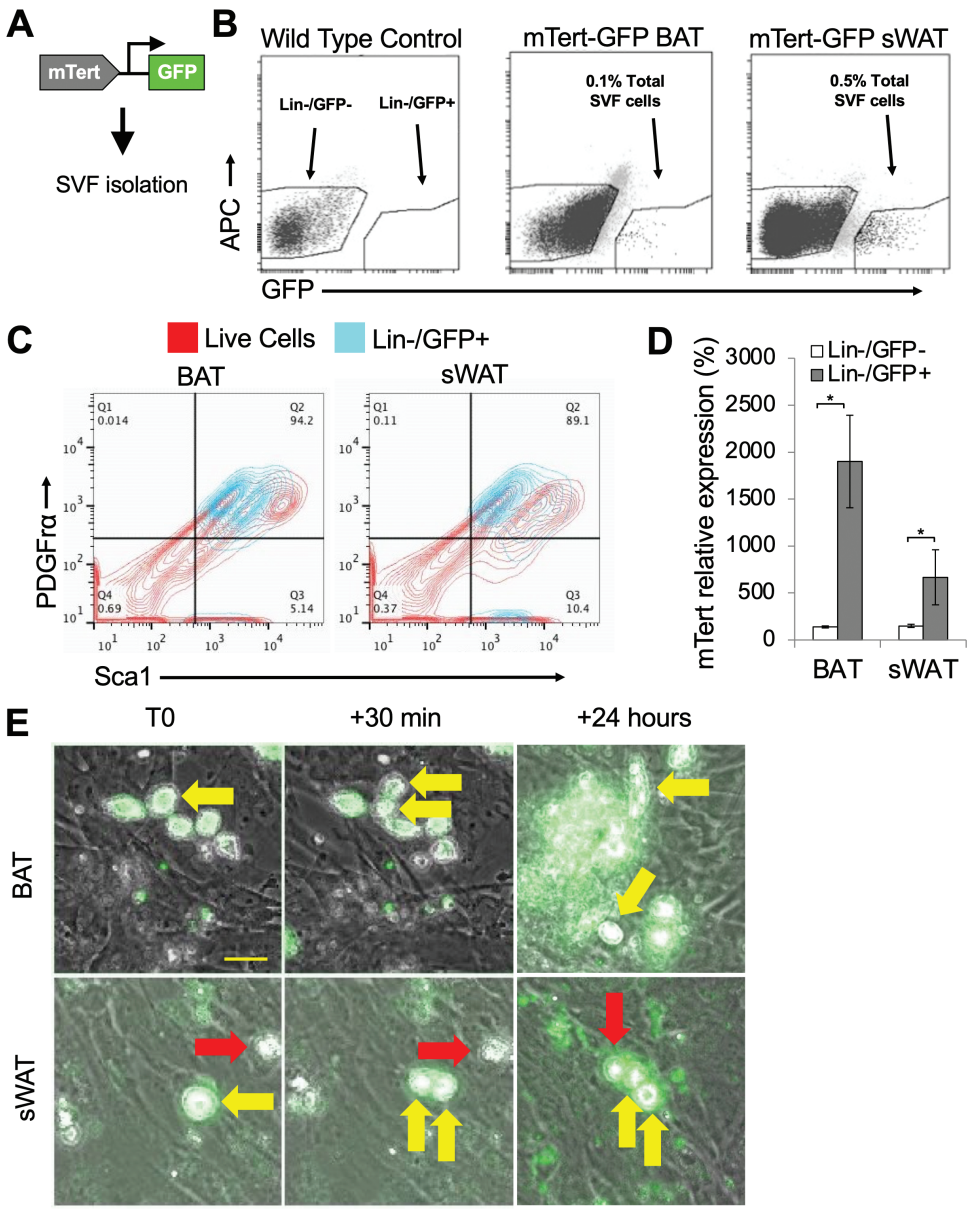

Figure 2. Cells that express mTert make up a subset of the adipogenic progenitor pool found in adipose tissue stromal vascular fraction (SVF). (**A**) Schematic diagram of transgene used to identify SVF cells that express mTert. (**B**) Example of GFP expression in mTert-GFP mouse SVF compared to wild-type control mice. In each panel, GFP fluorescence is on the x-axis and nonspecific staining is on the y-axis and the quantified populations of cells are marked. (**C**) Flow cytometry analysis of PDGFrα and Sca1 staining pattern of Lin-/GFP+ population compared to all other live cells in BAT and sWAT from mTert-GFP mice. In both charts, cells from a representative animal are shown. (**D**) mTert mRNA expression measured by qPCR in Lin-/GFP+ cells compared to Lin-/GFP-cells normalized to expression in whole tissue. Data are presented as mean ± SEM; $n = 5$ per group. $*P < .05$ for Student's t-test. (**E**) Representative time-lapse microscopy images immediately preceding mitosis of GFP+ cell, 30 minutes after cell division and 24 hours after division. Arrows mark the same original cell and each daughter cell. Data are representative of at least 3 mitotic events observed for each cell type. Scale bar is 200 μm.

these studies we combined mTert-rtTA::oTet-Cre mice with the Rosa26 membrane Tomato (mTom)/membrane GFP (mGFP) reporter line, to generate mTert-rtTA-mTmG mice, which expresses membrane-targeted Tomato at baseline and membrane-targeted GFP following Cre-mediated recombination. Next, SVF cells were then isolated from 10-week-old mice and cultured in the presence of doxycycline for 24 hours (Fig. 3B). Cells were then differentiated using a standard adipogenic induction/maturation cocktail (Fig. 3C).[12] After 11 days of adipogenic differentiation, GFP+ cells contained lipid droplets and stained positive for perilipin (Fig. 3C). We also subjected cultures from mTert-rtTA-mTmG mice to a neurogenic differentiation cocktail, which resulted in cells that stained positive for the neural marker Tuj1 (Supplementary Fig. 7B).[42] Taken together, these data indicate that mTert+ cells from BAT and WAT are capable of differentiation in vitro.

mTert-expressing Cells Proliferate in Response to HFD

To determine if mTert+ preadipocytes marked in adult animals could contribute to the long-term preadipocyte pool, mTert-rtTA-mTmG mice were treated with doxycycline for 24 hours at 10 weeks of age and chased until 20 weeks of age. Adipose

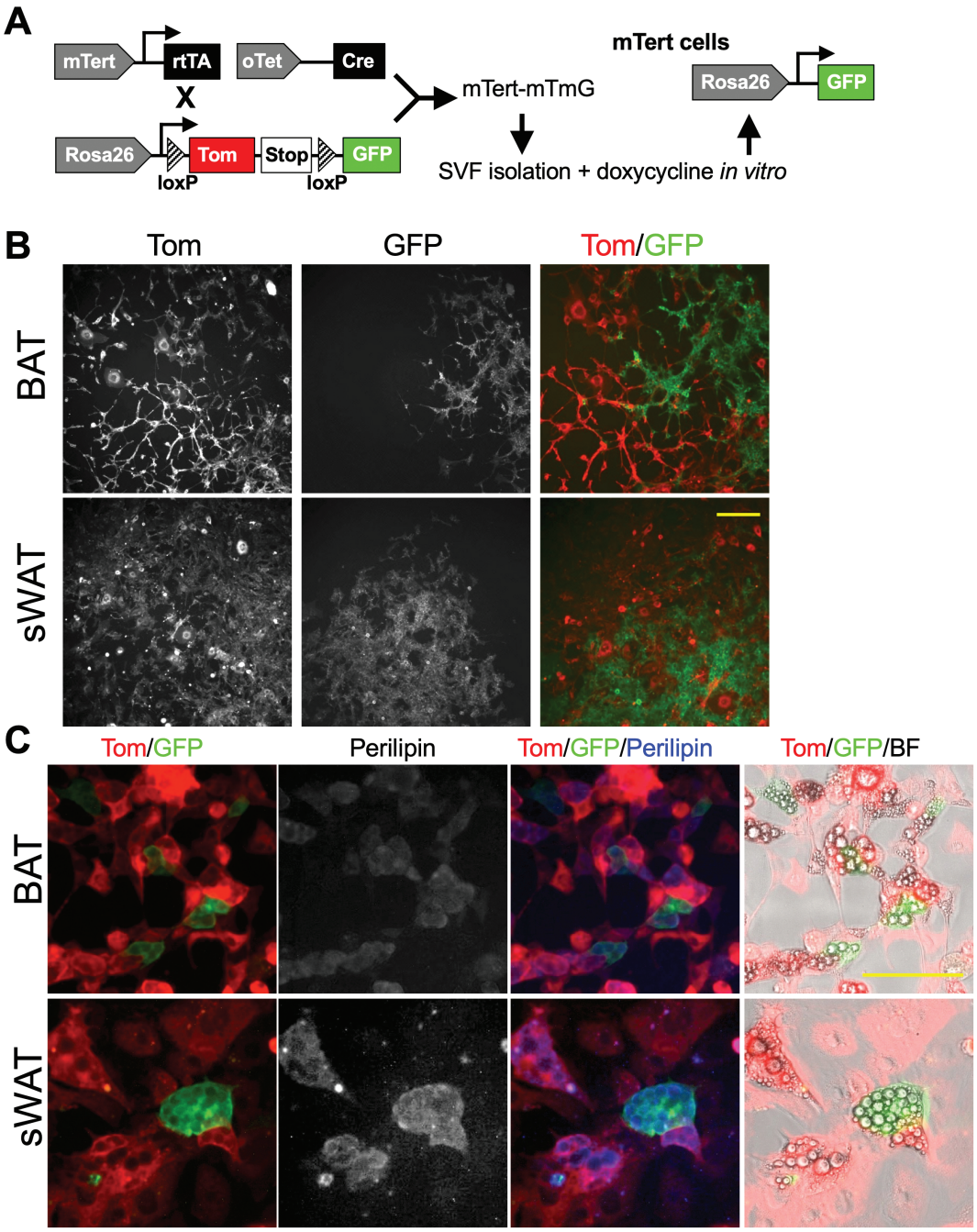

Figure 3. mTert+ cells can differentiate into adipocytes in vitro. (**A**) Schematic diagram of lineage-tracing strategy. The cellular lineage of mTert expressing cells was followed using a genetic reporter system wherein doxycycline induces expression of cre recombinase only in cells that express mTert, leading to recombination of mTert expressing cells and a change from red fluorescence to green fluorescence. (**B**) Fluorescent microscopy of mTert lineage-tracing stromal vascular fraction (SVF) cells from BAT and sWAT in undifferentiated state 24 hours after addition of doxycycline. Scale bar is 200 μm. (**C**) Fluorescent microscopy of mTert lineage-tracing SVF cells from BAT and sWAT after adipogenic differentiation and staining for perilipin (blue). Bright field (BF) image shows lipid droplet accumulation. Scale bar is 50μm.

tissue was isolated and mGFP+ preadipocytes in the SVF were quantified by flow cytometry (Fig. 4A). Approximately 1% (sWAT) and 5% (BAT) of stromal preadipocytes were mGFP+, indicating that they were derived from the mTert+ lineage-marked cells during the initial pulse of doxycycline (Fig. 4B). To investigate the adipogenic potential of mTert-expressing cells in vivo, mTert-rtTA-mTmG mice were treated with doxycycline for 24 hours after weaning to induce recombination, followed by a period of chase (Fig. 5A). Immediately after doxycycline treatment, we detected recombination in a rare population of individual small cells in BAT, and after 6 months of the chase, approximately 1% of the adipocytes expressed mGFP (Fig. 5B). In sWAT, recombination was also detected in a rare population of small cells after doxycycline treatment, and similar to BAT there were small, rare adipocytes labeled after 6 months of chase (Fig. 5C).

High-fat diet (HFD) is known to promote hypertrophy and hyperplasia of adipose tissue.[43,44] To test the hypothesis

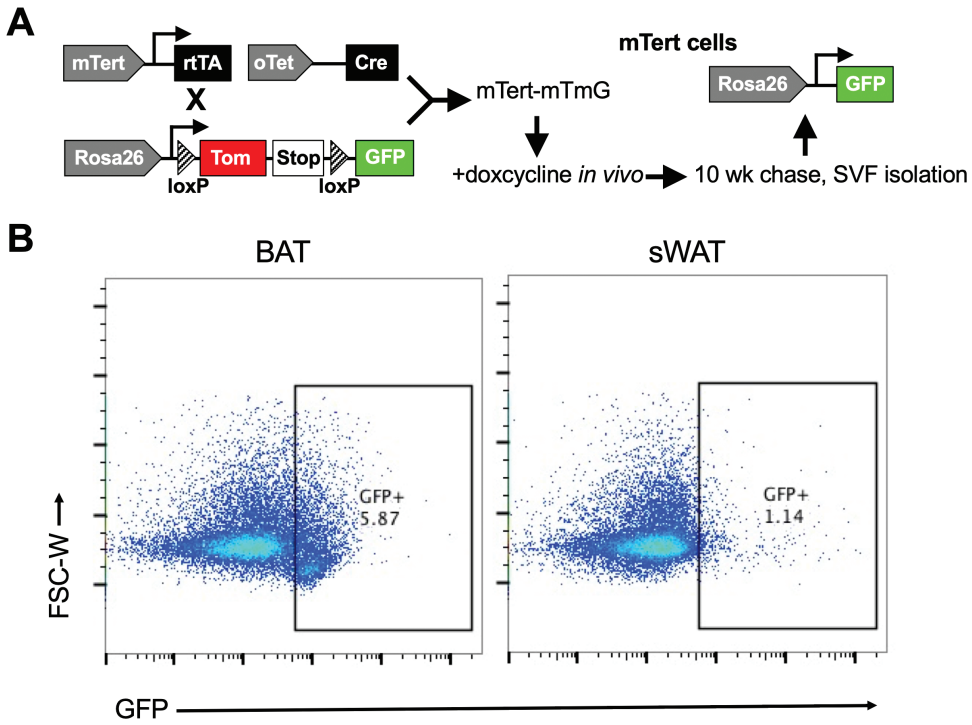

Figure 4. Tracing the mTert lineage in preadipocytes in vivo. (**A**) The cellular lineage of mTert expressing cells was followed in vivo using a genetic reporter system to pulse animals with doxycycline to induce expression of cre recombinase only in cells that express mTert, leading to recombination of mTert expressing cells and a change from red fluorescence to green fluorescence. This was followed by a chase period after which animals were sacrificed and stromal vascular cells were analyzed by flow cytometry. (**B**) Flow cytometry analysis of preadipocytes isolated from the stromal vascular fraction of BAT and sWAT from mice pulsed for 1 day with doxycycline and then chased for 22 weeks. Forward scatter (FSC-W) is on the y-axis and GFP expression is on the x-axis with the percentage of preadipocytes staining above background indicated.

that mTert cells in adult mice are activated by nutrient excess, 18-week-old mTert-rtTA-mTmG mice were treated with doxycycline for 24 hours to indelibly label all mTert+ cells, then the mice were fed HFD for 5 weeks to induce adipogenesis. By counting GFP+ adipocytes in mice fed HFD compared to litermates fed a chow diet, the contribution of the mTert lineage to newly formed adipocytes could be quantified. We observed an increased fraction of large, lipid-laden adipocytes derived from the mTert lineage in HFD-fed mice compared to controls (Fig. 5D). Histological analysis also showed an approximately 5-fold increase in the number of mGFP-positive cells relative to mTom positive cells in mice fed HFD compared to chow-fed animals. We did not observe the expansion of the mTert lineage into mature adipocytes in BAT in response to HFD (Supplementary Fig. 8A). Cold exposure can also stimulate the recruitment of new adipocytes in BAT; however, we did not observe any newly formed adipocytes from the mTert-positive lineage in BAT from 18-week-old mTert-rtTA-mTmG mice that were treated with doxycycline for one week and then housed at 5°C for 1 week (Supplementary Fig. 8B). These results indicate that mTert cells can be induced to differentiate in sWAT by HFD; however, their contribution to the mature adipocyte pool in other depots remains unclear.

Discussion

By indelibly labeling mTert cells in adult animals and following the development of this lineage in vivo using an inducible bioluminescent mTert lineage-tracing strain, we were able to take an unbiased approach to examine the contribution of these putative tissue-resident stem cells to whole-body tissue development. mTert lineage-tracing animals allowed us to not only identify new locations where tissue is derived from the mTert lineage but further allowed us to serially examine the same animal longitudinally so that subtle differences in the localization of signal could be compared between time points. Importantly, mice were pulsed with doxycycline for only a single day to label cells, however, recombined cells could still be detected more than a year after the initial recombination event, showing the long-term contribution of these cells and their progeny that is consistent with reports of mTert as a stem cell marker.[5,7,8,36] Their behavior in vitro mimicked the phenotypes that we observed in vivo, as the cells were capable of division and adipogenic differentiation in vitro. In addition to the labeling we observed in the adipose tissue, the luminescent signal was observed in other locations such as the liver, intestine, and brain and may correspond to stem/progenitor cells in one of the well-studied niches or migratory streams of these organs, and warrants further investigation.[38,45]

Because we observed long-term labeling in BAT and sWAT, we focused on these tissues in the current study. Adipose tissue development begins before birth[46] and weaned animals are thought to have fully mature adipose tissue depots. To identify the specific cellular subpopulations that regulate and undergo adipogenesis in vivo, previous studies have utilized lineage-tracing approaches with transgenic mice using Cre/Lox technology to indelibly mark genetically defined cell types.[47] By marking the cells at different developmental time points and then examining their fate at different ages, genes that identify adipose tissue progenitor cells, such as PDGFrα,[13]

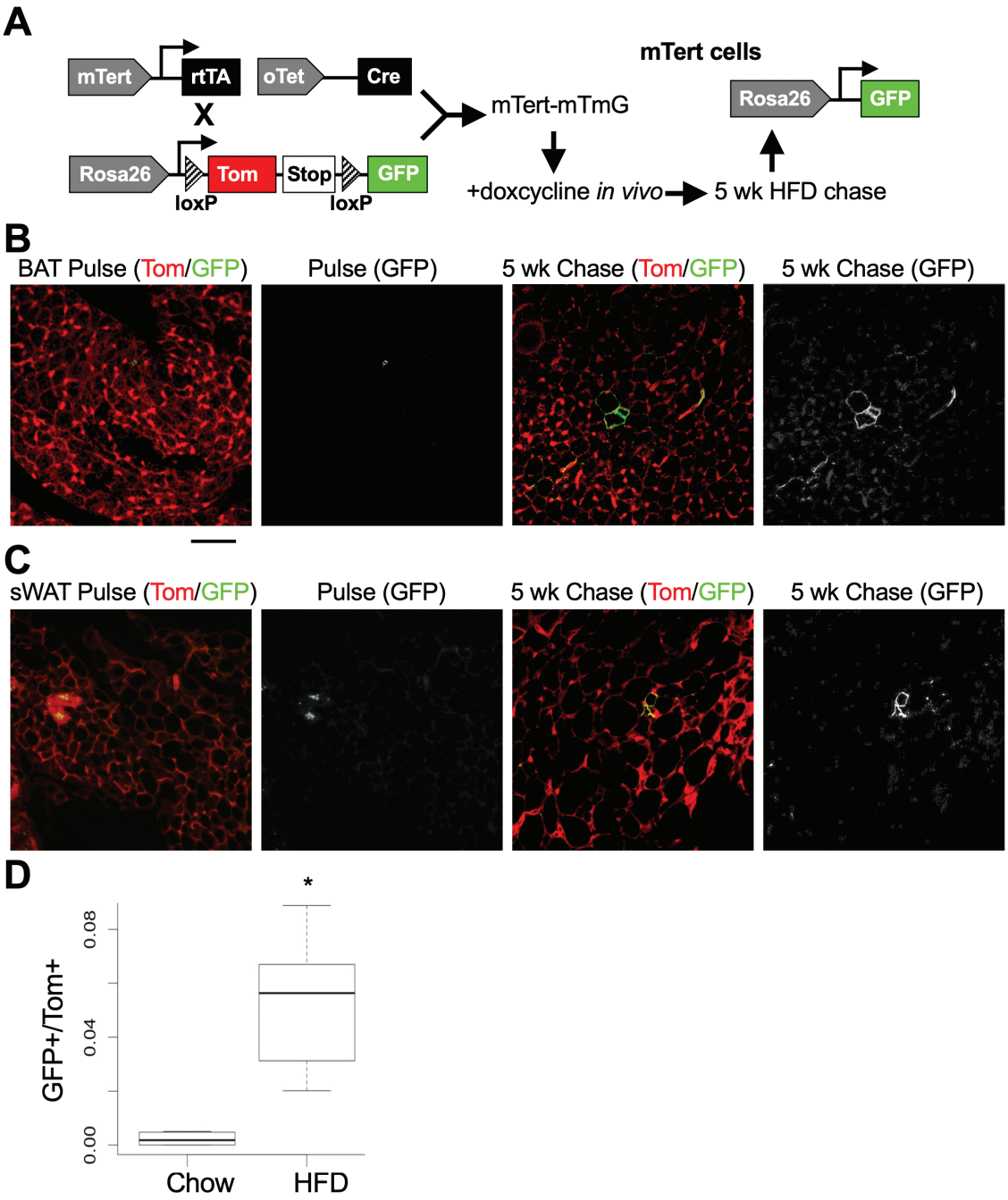

Figure 5. Tracing the mTert lineage into adipocytes in vivo (**A**) The cellular lineage of mTert expressing cells was followed in vivo using a genetic reporter system to pulse animals with doxycycline to induce expression of cre recombinase only in cells that express mTert, leading to recombination of mTert expressing cells and a change from red fluorescence to green fluorescence. This was followed by a chase period during which some mice were challenged with HFD. (**B**) Fluorescent microscopy of BAT from mTert lineage-tracing mice after 24-hour pulse of doxycycline and 6-month chase period. Each image is representative of 4-6 animals per group. Scale bar is 200 μm. (**C**) Fluorescent microscopy of sWAT from mTert lineage-tracing mice after 1 week pulse of doxycycline and 6-month chase period. Each image is representative of 4-6 animals per group. Scale bar is 200 μm. (**D**) GFP+ and Tom+ adipocytes were counted in adipose tissue micrographs and expressed as a ratio. Data are means ± SEM (whiskers) with highest and lowest outliers marked by the rectangle; *n* = 5 mice with at least 3 sections per mice in each group.

PDGFrβ,[43] Sca-1,[12,16] CD34,[14], CD29, and CD24[15,16] have been identified. Interestingly, not all mature adipocytes within each depot arise from common lineages. For example, expression of the myogenic factor Myf5 marks the progenitor cells for approximately 95% of brown adipocytes, but only 5% of adipocytes in sWAT arise from a Myf5 lineage, showing the heterogeneity both within depots and between them.[21,23,48] We detected a rare population of stromal cells in both adipose tissue depots that expressed putative progenitor markers and gave rise to mature adipocytes, suggesting that this lineage of cells shared between the 2 tissues may be specialized in function.

Adipogenic differentiation of precursor cells into mature adipocytes occurs both during the routine turnover of older cells and in response to pro-adipogenic cues.[49,50]. One function of white adipose tissue is to expand in response to excess

energy to increase storage capacity, although the expansion of sWAT is thought to be largely driven by hypertrophy with limited hyperplasia.[44,50]. We detected depot-specific expansion of the mTert cellular lineage in sWAT of mice fed with HFD, with no expansion of this lineage in BAT, suggesting mTert lineage mature adipocytes represent the new population of adipocytes recruited in sWAT by HFD. It will be interesting to compare adipocytes from the mTert lineage to adipocytes from the non-mTert lineage functionally. Cold temperature can be a pro-adipogenic stimuli in BAT[43] and sWAT, where it activates the recruitment of beige adipocytes.[51] We did not detect the expansion of the mTert lineage in BAT or sWAT tissue from mice exposed to cold, suggesting the mTert lineage does not contribute to browning and the cues that activate the mTert cellular lineage in BAT require further investigation.

Using a putative stem cell marker such as mTert to track the cellular lineages of progenitor cells into different tissues represents a reverse genetics approach toward understanding cellular heterogeneity. Single-cell sequencing has recently become the preferred forward genetics approach and has uncovered both genetic and functional heterogeneity between preadipocytes in the same fat depot.[52,53] As previously described, mTert+ stromal cells express the putative preadipocyte markers PDGFrα/β, Sca-1, CD24, and CD29; however, they are only a small percentage of the total population of cells that express these markers, suggesting they are a distinct cell type. Currently, human adipose stem cell therapy has shown promise as a treatment for a variety of different diseases.[54,55] and conditions.[56] and further understanding of the different subpopulations of cells residing in adipose SVF may improve the efficacy of these treatments.

Conclusion

The ability to localize and purify functional progenitor cells is essential to fully understand how any tissue or organ is formed and maintained so the mouse models reported herein will significantly contribute to our understanding of the stem cells that contribute to these processes. Because mTert expression marks putative adult tissue-resident stem cells in many tissues, the therapeutic potential of these cells should be studied further. Given the current epidemic of obesity and related disorders, this need is particularly acute for adipose tissues making the identification of mTert-expressing stem cells that are induced to differentiate into adipocytes by HFD an important discovery.

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Conflict of Interest

K.L.T. declared employment with Neuright, Inc. (serving as part time CSO). **Y-H.T.** declared advisory role with Cellarity, Inc. The other authors indicated no financial relationships.

Author Contributions

Conception and design, collection and/or assembly of data, data analysis and interpretation, manuscript writing, and final approval of manuscript: M.D.L. Conception and design, collection and/or assembly of data, manuscript writing, and final approval of manuscript: D.L.C. Manuscript writing and final approval of manuscript: K.L.T. Conception and design, financial support, manuscript writing, and final approval of manuscript: D.T.B. Conception and design, financial support, manuscript writing, and final approval of manuscript: Y.-H.T.

Data Availability

The data underlying this article are available in the article and in its online supplementary material.

Supplementary Material

Supplementary material is available at *Stem Cells* online.

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