RESEARCH PAPER

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Characterization of Cre recombinase models for the study of adipose tissue

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The study of adipose tissue in vivo has been significantly advanced through the use of genetic mouse models. While the aP2-Cre^{BI} and aP2-Cre^{Salk} lines have been widely used to target adipose tissue, the specificity of these lines for adipocytes has recently been questioned. Here we characterize Cre recombinase activity in multiple cell populations of the major adipose tissue depots of these and other Cre lines using the membrane-Tomato/membrane-GFP (mT/mG) dual fluorescent reporter. We find that the aP2-Cre^{BI} and aP2-Cre^{Salk} lines lack specificity for adipocytes within adipose tissue, and that the aP2-Cre^{BI} line does not efficiently target adipocytes in white adipose depots. Alternatively, the Adiponectin-CreERT line shows high efficiency and specificity for adipocytes, while the PdgfRα-CreERUCL and PdgfRα-CreERJHU lines do not efficiently target adipocyte precursor cells in the major adipose depots. Instead, we show that the PdgfRα-Cre line is preferable for studies targeting adipocyte precursor cells in vivo.

Introduction

Adipose tissue is recognized as a vital player in the maintenance of energy balance, nutrient status, and metabolic homeostasis.[1] White adipose tissue (WAT) retains the ability to grow and shrink dramatically to meet the energetic needs of an organism; however, severe metabolic consequences can result from excessive WAT gain, the defining characteristic of obesity, or extreme loss of WAT mass, known as lipodystrophy.[2,3] These include defects in glucose homeostasis, inflammation, and cardiovascular function.[3-6] In order to fully understand these complex multi-organ pathologies, studies must be performed in vivo, and our ability to address the role of adipose tissue in these systems relies on the tools that have been developed to manipulate gene expression within adipose tissue in vivo.

Mature adipocytes compose the majority of the volume of adipose tissue, and the remaining cell populations in the tissue include blood cells, endothelial cells, various immune cell populations, and adipocyte precursors. Recently, the identification of specific murine white adipocyte precursor populations, including Lin$^-$:CD29$^+$:CD34$^+$:Sca-1$^+$:CD24$^+$ adipocyte progenitor cells and Lin$^-$:CD29$^+$:CD34$^+$:Sca-1$^+$:CD24$^-$ preadipocytes,[7] has enabled further study of the adipocyte lineage in vivo. Furthermore, several groups have reported that platelet-derived growth factor receptor α (PdgfRα) is expressed on adipocyte precursor cells in WAT and traces all adipocytes in normal murine WAT depots,[8,9] which indicates that this promoter is a useful tool for targeting the adipocyte cellular lineage.

The Cre/lox system of gene targeting has revolutionized the study of tissue-specific function in vivo. Cre recombinase can be integrated downstream of an endogenous promoter, often termed a "knock-in", or it can be placed under control of a promoter fragment which is then integrated into the genome at a random site. The length of this promoter sequence and the location of the integration site can affect the expression pattern of the Cre transgene, and may affect the fidelity of Cre expression compared with the expression of the endogenous gene.[10] Additionally, temporal control over gene expression can be achieved with inducible targeting models such as doxycycline-regulated Cre expression or tamoxifen-sensitive Cre. When applying these techniques, however, it is important to keep in mind the limitations and pitfalls of these approaches including the erroneous expression of Cre transgenes, the varying sensitivity of different genomic sites to Cre-mediated loxP recombination, and the potential for changes in Cre expression or efficacy over several generations of mouse colony maintenance.[11,12]

To facilitate the study of adipocyte function in physiologically relevant contexts, several adipocyte-specific promoters have been generated to drive the expression of Cre recombinase in adipose tissues. Of these, the most commonly used Cre transgenes

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were created from the promoter of the fatty acid binding protein 4 (*Fabp4*) gene which encodes adipocyte protein 2 (aP2).[13] Two separate lines (aP2-Cre^{BI} and aP2-Cre^{Salk}) were generated[14,15] and widely used in studies to target adipocytes in vivo.[16] However, these lines have been shown to have Cre activity in other tissues and cell types, including brain, endothelial cells, macrophages, adipocyte precursors and embryonic tissues.[16-19] Alternatively, the promoter of the adipocyte-specific protein Adiponectin (encoded by *Adipoq*) has been used to generate Cre lines for the study of adipocyte function.[20,21] These Adiponectin-Cre mouse lines have been shown by multiple groups to be highly specific to adipose tissue.[16,18,20] To identify the most useful tools for targeting the adipocyte lineage in vivo, we sought to investigate the recombination efficiency and specificity of commonly used Cre lines, using a membrane-targeted dual fluorescent reporter model to quantitatively assess Cre recombinase activity in adipose tissues.

Here we characterize the recombination of specific cell populations within adipose tissue in the aP2-Cre^{BI} and aP2-Cre^{Salk} lines, as well as the tamoxifen-inducible Adiponectin-CreERT and PdgfRα-CreERT lines. We find incomplete targeting of adipocytes and a lack of specificity with the aP2-Cre^{BI} and aP2-Cre^{Salk} lines, and remarkably low recombination efficiency in adipocyte precursors in PdgfRα-CreER lines. Finally, we show that the Adiponectin-CreERT is a useful inducible model for targeting mature adipocytes, while the PdgfRα-Cre is useful for studying the adipocyte lineage in vivo.

Results

To characterize the pattern of Cre expression in the aP2-Cre lines, we crossed each of these lines to the dual fluorescent reporter model (mT/mG) that we have previously used to perform lineage tracing of white adipose tissue.[9,22] This reporter expresses membrane-targeted tdTomato (mT), and the expression of Cre recombinase results in the excision of the tdTomato cassette, which then permits the expression of membrane-targeted eGFP (mG). Since these fluorescent proteins are membrane-targeted, this model provides clear fluorescently labeled cellular boundaries, facilitating the identification of adipocytes in which Cre-mediated recombination has occurred. When we analyzed the pattern of Cre expression in several adipose depots of aP2-Cre^{BI}; mT/mG mice, we found that few adipocytes were labeled in WAT depots, while approximately half of the adipocytes in the intrascapular brown adipose tissue (iBAT) depot were labeled (**Fig. 1A and B**). Additionally, flow cytometry analysis indicated that a large percentage of endothelial cells were labeled in both WAT and BAT depots in the aP2-Cre^{BI} line, while the adipocyte precursor populations were not labeled in WAT depots (**Fig. 1B**). Further confocal analysis confirmed that the majority of eGFP-positive cells in SWAT co-stained with the endothelial stain GSIB$_4$ (**Fig. 1C**). Finally, we observed negligible labeling of both liver and skeletal muscle cells of these mice (data not shown). These data indicate that while this line was originally shown to efficiently label adipocytes,[14] it now targets primarily brown adipocytes and endothelial cells within adipose tissue.

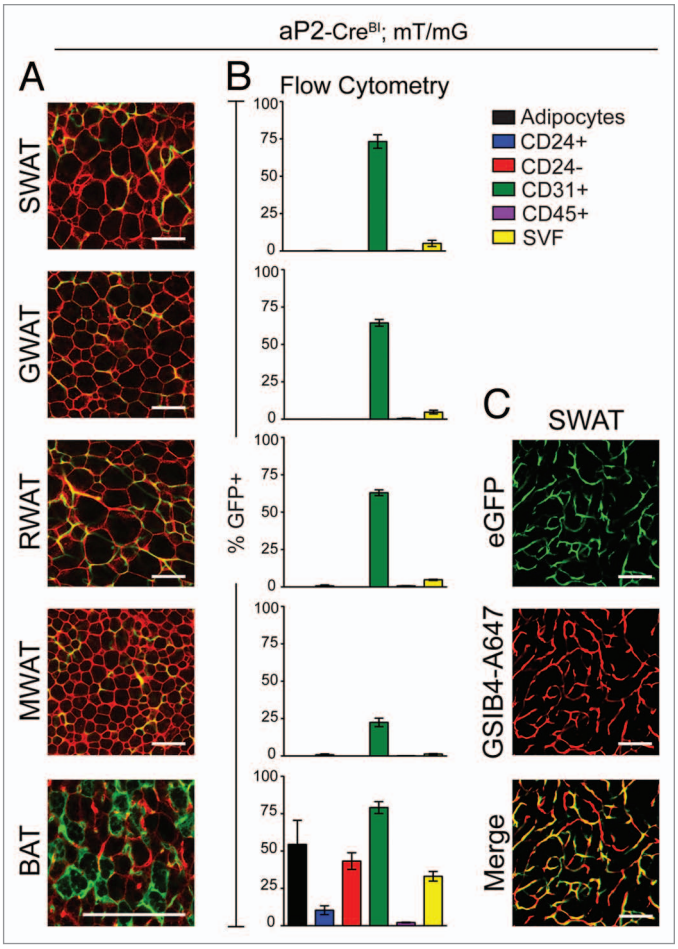

Figure 1. aP2-Cre^{BI} primarily labels endothelial cells and brown adipocytes. (**A**) Confocal microscopy of the indicated adipose tissue depots from aP2-Cre^{BI}; mT/mG mice. (**B**) Quantification of cells displaying Cre-mediated expression of eGFP in cell populations of the indicated adipose tissue depots (n = 3). (**C**) Confocal microscopy of SWAT from aP2-Cre^{BI}; mT/mG mice stained with isolectin GSIB$_4$ to label endothelial cells. Scale bars in (**A and C**) are 100 μm. SWAT, subcutaneous WAT; GWAT, gonadal WAT; RWAT, retroperitoneal WAT; MWAT, mesenteric WAT; BAT, brown adipose tissue; SVF, stromal vascular fraction.

We next characterized Cre expression in the aP2-Cre^{Salk} line using the mT/mG fluorescent reporter. We found that the percentage of adipocytes labeled by this line in WAT depots was much higher than the aP2-Cre^{BI} line, and varied between 50 and 80 percent (**Fig. 2A and B**). Brown adipocytes were labeled to a similar degree (**Fig. 2A and B**). While there was some labeling in the adipocyte precursor populations in all depots, we also observed some labeling in blood lineage cells and significant labeling of CD31$^+$ endothelial cells in all adipose depots analyzed (**Fig. 2B**), again indicating a lack of specificity for the adipocyte lineage in the aP2-Cre^{Salk} line. However, we observed negligible Cre-mediated recombination in cells of the liver and skeletal muscle of these mice (data not shown).

The promoter for adiponectin, a hormone secreted by mature adipocytes, has been used by multiple groups to

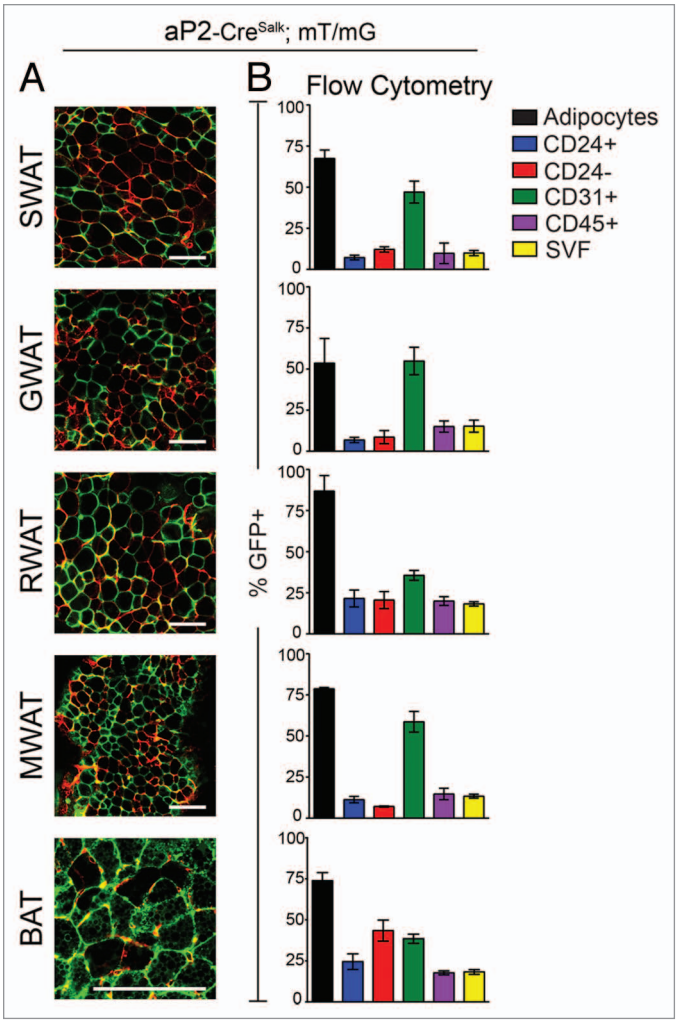

Figure 2. aP2-Cre^{Salk} labels multiple cell populations within adipose tissue. (**A**) Confocal microscopy of the indicated adipose tissue depots from aP2-Cre^{Salk}; mT/mG mice. (**B**) Quantification of cells displaying Cre-mediated expression of eGFP in cell populations of the indicated adipose tissue depots (n = 3). Scale bars in (**A**) are 100 μm. SWAT, subcutaneous WAT; GWAT, gonadal WAT; RWAT, retroperitoneal WAT; MWAT, mesenteric WAT; BAT, brown adipose tissue; SVF, stromal vascular fraction.

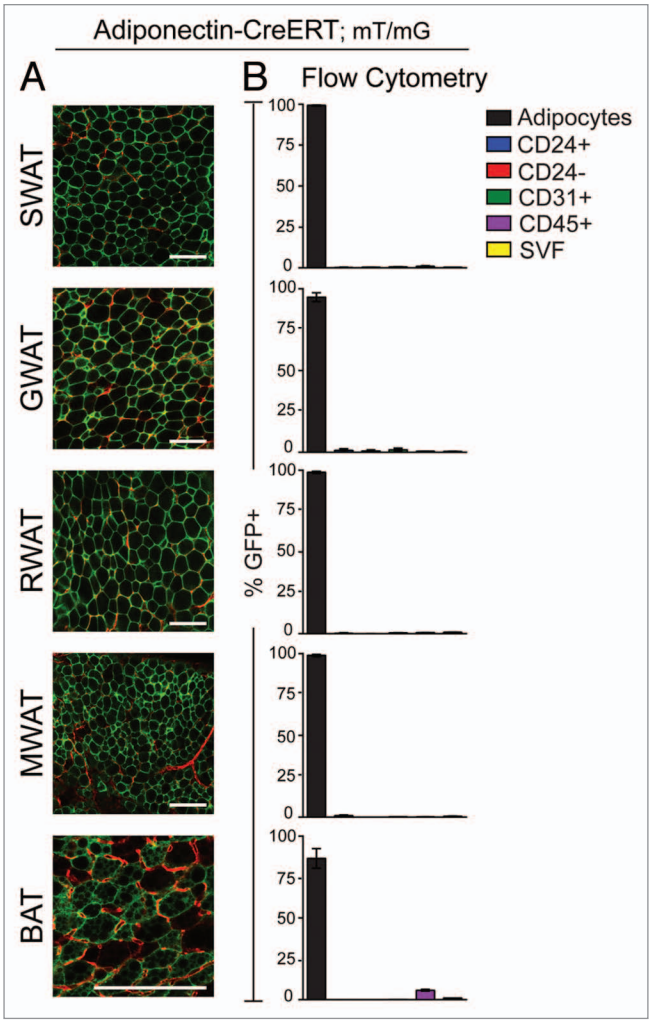

Figure 3. Adiponectin-CreERT labels mature adipocytes. (**A**) Confocal microscopy of the indicated adipose tissue depots from Adiponectin-CreERT; mT/mG mice after tamoxifen treatment. (**B**) Quantification of cells displaying Cre-mediated expression of eGFP in cell populations of the indicated adipose tissue depots (n = 3). Scale bars in (**A**) are 100 μm. SWAT, subcutaneous WAT; GWAT, gonadal WAT; RWAT, retroperitoneal WAT; MWAT, mesenteric WAT; BAT, brown adipose tissue; SVF, stromal vascular fraction.

generate adipocyte-specific Cre lines.[20,21] The most widely used Adiponectin-Cre^{R} line[12] has been shown several times to be specific to adipocytes,[9,16,18] and useful for targeting mature adipocytes in vivo. To characterize inducible Cre expression in a new Adiponectin-CreER line generated with the same BAC used to create the Adiponectin-Cre^{R} mice, we crossed this line to the mT/mG reporter line and treated mice with 50 mg/kg tamoxifen for 6 d. Before tamoxifen treatment, no adipocyte labeling was observed (**Fig. S1A**), and after tamoxifen treatment, adipocyte recombination was nearly 100% in all WAT depots analyzed, with negligible recombination in any other cell population, and greater than 85% recombination was observed in brown adipocytes (**Fig. 3A and B**). Importantly, no cells within the adipose SVF displayed Cre-mediated recombination in this model, including the adipocyte precursor populations, (**Fig. 3B**), confirming the specificity of this promoter for mature adipocytes.

We and other groups have shown that PdgfRα is expressed on adipocyte precursor populations within WAT,[8,9] and that almost all of the adipocyte precursors are labeled in PdgfRα-Cre; mT/mG mice. In addition, the vast majority of the cells displaying Cre-recombination within WAT SVF of PdgfRα-Cre; mT/mG mice are adipocyte precursors.[9] To determine whether this Cre line may be useful for metabolic studies targeting the adipocyte lineage, but not other major metabolic tissues, we assessed Cre-mediated recombination in the cells of the muscle and liver of PdgfRα-Cre; mT/mG mice. We found low levels of recombination in both of these tissues (**Fig. 4A**), indicating that the muscle and liver are minimally targeted by the PdgfRα promoter. These

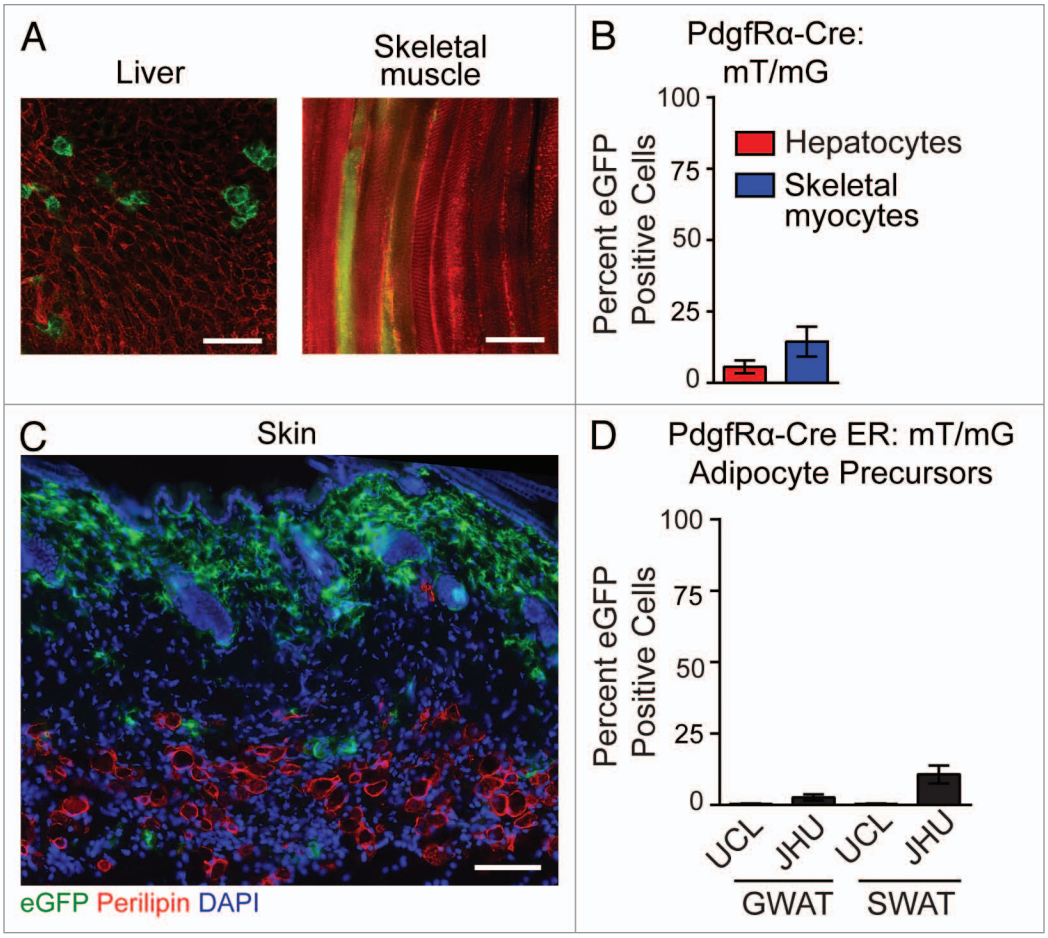

Figure 4. PdgfRα-CreER does not efficiently label adipocyte precursor cells. (**A**) Confocal microscopy of the liver and skeletal muscle (gastrocnemius) from PdgfRα-Cre; mT/mG mice. (**B**) Quantification of cells displaying Cre-mediated expression of eGFP in liver and skeletal muscle of PdgfRα-Cre; mT/mG mice ($n = 3$). (**C**) Immunofluorescence image of the back skin of PdgfRα-CreER^{UCL}; mT/mG mouse after tamoxifen injection showing recombination in fibroblastic cells of the upper dermis. Perilipin staining of adipocytes indicates the location of the adipocyte layer within the dermal compartment. Representative image from 2 animals. (**D**) Quantification of cells displaying Cre-mediated expression of eGFP in the adipocyte precursor population of the indicated depots of PdgfRα-CreER^{UCL}; mT/mG and PdgfRα-CreER^{JHU}; mT/mG mice after daily treatment of 50mg/kg tamoxifen for 6 d ($n = 3–4$). Scale bars in (**A and C**) are 100 μm. SWAT, subcutaneous WAT; GWAT, gonadal WAT; SVF, stromal vascular fraction.

data suggest that the PdgfRα-Cre line may be appropriate for functional studies of the adipocyte cellular lineage in vivo.

Inducible versions of the PdgfRα-Cre mouse line have been generated by both the Richardson group[23] (PdgfRα-CreER^{UCL}) and the Bergles group[24] (PdgfRα-CreER^{JHU}). Both of these lines have previously been shown to label oligodendrocyte precursors in the brain.[23,24] The PdgfRα-CreER^{UCL} line has been used to label adipocyte lineage cells in WAT[8] and fibroblast-like cells in the skin,[25] although with variable efficiency. To assess the potential utility of both of these lines for the study of adipocyte precursors in WAT depots in vivo, we treated PdgfRα-CreER^{UCL}; mT/mG and PdgfRα-CreER^{JHU}; mT/mG mice with 50 mg/kg tamoxifen daily for 6 d and subsequently analyzed the percentage of adipocyte precursor cells displaying Cre-mediated recombination in the skin and primary WAT depots of these mice. In tamoxifen-treated PdgfRα-CreER^{JHU}; mT/mG mice, we observed a high level of GFP-positive cells in the dermal layer of the skin (**Fig. 4B**), a region known to contain PdgfRα-Cre-expressing cells including an adipogenic population;[25,26] however, less than 1% of the intradermal adipocyte precursor cells were labeled (data not shown). When we analyzed the stromal vascular fraction of WAT depots in PdgfRα-CreER^{UCL}; mT/mG mice, we observed very low rates of recombination (less than 3%) in adipocyte precursor populations of both GWAT and SWAT, with only marginally better recombination percentages in the SWAT of PdgfRα-CreER^{JHU}; mT/mG mice (**Fig. 4C**). Treatment of PdgfRα-CreER^{JHU}; mT/mG mice with a higher tamoxifen dose (300 mg/kg for 5 d), did not improve the recombination efficiency (**Fig. S1C**), indicating that Cre activity in adipocyte precursors in these lines is insufficient for assessing gene function in adipocyte precursors in vivo.

Discussion

Tools for the genetic targeting of adipose tissue in vivo are essential for the study of adipose function and metabolic disease.

Consistent with previous reports,[16,17] our data show that the aP2-Cre^{BI} line and the aP2-Cre^{Salk} line are not specific to adipocytes. However, contrary to previous studies using cytoplasmic LacZ cre reporters,[18] quantitative analysis of adipocyte labeling with the mT/mG reporter indicates that the aP2-Cre^{BI} line does not label the majority of adipocytes in WAT depots. Since this could be due to a shift in the Cre expression pattern in these lines over generations,[12] these data emphasize the importance of monitoring recombination within a line over time. Additionally, these data highlight the importance of determining the specificity of Cre-recombination to the cell types of interest in a particular tissue in studies of cellular lineage dynamics and cell function.

We and others have previously found that the Adiponectin-Cre^{R} effectively targets mature adipocytes,[9,16,18,20] and we show here that the Adiponectin-CreER line can be used to efficiently target adipocytes in an inducible manner. This model can be applied in studies of adult animals in which developmental defects in adipose tissue could present confounding effects, and also provides the temporal control necessary to perform quantitative pulse-labeling studies when coupled with the mT/mG reporter.

The PdgfRα-CreER model would also be a valuable tool for targeting adipocyte precursor cells. While one group has used very high doses of tamoxifen to achieve partial recombination in WAT-resident APs,[8] we show here that in our hands neither of the existing PdgfRα-CreER lines efficiently label this population, despite labeling of other PdgfRα-expressing populations in the dermis (**Fig. 4**). Given that both of the promoter sequences used to generate the PdgfRα-CreER lack a region of at least 60 kb upstream of the PdgfRα gene compared with the PdgfRα-Cre construct, this low labeling efficiency may be due to the lack of *cis*-regulatory elements that are normally necessary to drive expression from the PdgfRα promoter in certain cell populations.[23,24,27] Another potential factor in the difference in labeling between the PdgfRα-CreER and PdgfRα-Cre lines is differences in integration sites in the genome. Regardless of the reason, the low labeling efficiency in these PdgfRα-CreER lines indicates that they are not useful for quantitative adipocyte lineage studies or for study of gene function in the adipocyte lineage.

Even when the promoter region used to drive transgene expression is large, differences in enhancer activity or positional effects of the insertion site can cause the expression of the transgene to differ from the endogenous gene. This appears to be the case for the PdgfRα-Cre line, which efficiently labels adipocyte precursors.[9] During murine embryonic development, staining for endogenous PdgfRα and experiments utilizing a knock-in fluorescent reporter have shown that PdgfRα is expressed in regions of the endoderm, mesoderm and ectoderm, with the most prominent expression occurring in mesodermal tissues.[28-31] Starting at embryonic day 8, PdgfRα is expressed throughout the somites,[28] which give rise several mesodermal tissues including the skeletal muscle, dermis, and cartilage. However, in adult PdgfRα-Cre mice, the broad recombination that would be expected given this embryonic expression pattern is not observed. For example, adult skeletal muscle displays a low level of Cre-mediated recombination in the PdgfRα-Cre line. These data suggest that the expression of Cre from the PdgfRα promoter in the PdgfRα-Cre model does not mimic these early embryonic expression patterns (**Fig. 4**). This restricted expression may be due to a lack of promoter and/or enhancer elements in the PdgfRα-Cre genetic construct that are required to induce embryonic expression of the gene. The PdgfRα-Cre model has been shown to label other cell populations such as the Muller glial cells of the retina,[27] but our data show that it is not significantly expressed in the parenchymal cells of other major metabolic organs such as the liver and skeletal muscle. These findings indicate that PdgfRα-Cre should not have significant "off target" effects in other metabolic tissues. However, the known Cre expression from this promoter in other cell types, such as oligodendrocytes,[23] should be considered when interpreting results from this model.

Another caveat that must be considered when using either the PdgfRα or the Adiponectin promoters for the study of adipose function is that these models express Cre recombinase in both white and brown adipocytes. There are not currently any models known to specifically target WAT without targeting BAT, and the promoters known to target BAT also target either muscle and subsets of white adipocytes[32-34] or target both beige and brown adipocytes.[35] Therefore, we conclude that PdgfRα-Cre is currently the best model available for the targeting of adipocyte lineage cells in adipose depots in vivo, while the Adiponectin-Cre^{R} and Adiponectin-CreER are effective for targeting mature adipocytes.

Materials and Methods

All animal studies followed guidelines issued by Yale University's Institutional Animal Care and Use Committee (IACUC). aP2-Cre^{BI} mice were a generous gift from Dr Barbara Kahn, obtained in October 2013. The PdgfRα-CreER^{UCL} mouse line[23] was independently obtained from Dr Anne Perl and Dr Dana McTigue. The aP2-Cre^{Salk} mice (005069), PdgfRα-Cre mice (013148), PdgfRα-CreER^{JHU} mice (018280), and mT/mG mice (007676) were purchased from Jackson Laboratories. AdiponectinCre-ERT mice (024671) are now available at Jackson Laboratories. Except for the Adiponectin-CreERT and PdgfRα-CreER lines, all mice analyzed were males between 4 and 6 wk of age.

For Adiponectin-CreERT experiments, 8-wk-old male mice were given daily intraperitoneal injections of 50 mg/kg tamoxifen in vegetable oil for 6 d. Mice were then allowed to recover for one week, and then sacrificed. For PdgfRα-CreER experiments, where indicated, mice between 5 and 10 wk of age were given either intraperitoneal injections of 50 mg/kg tamoxifen in vegetable oil daily for 6–7 d or oral gavage of 300 mg/kg tamoxifen in vegetable oil daily for 5 d.

Confocal microscopy and flow cytometry were performed as described.[9,36,37] For immunofluorescence, 14 μm skin sections were fixed with 4% paraformaldehyde and incubated with primary antibodies against GFP (chicken, Abcam, 1:1000) and perilipin A (goat, Abcam, 1:1000) overnight at 4 °C followed by incubation with alexa fluor-conjugated secondary antibodies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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Supplemental Materials

Supplemental materials may be found here:
www.landesbioscience.com/journals/adipocyte/article/29674/

References

1. Rosen ED, Spiegelman BM. Adipocytes as regulators of energy balance and glucose homeostasis. Nature 2006; 444:847-53; PMID:17167472; http://dx.doi.org/10.1038/nature05483
2. Després JP, Lemieux I, Bergeron J, Pibarot P, Mathieu P, Larose E, Rodés-Cabau J, Bertrand OF, Poirier P. Abdominal obesity and the metabolic syndrome: contribution to global cardiometabolic risk. Arterioscler Thromb Vasc Biol 2008; 28:1039-49; PMID:18356555; http://dx.doi.org/10.1161/ATVBAHA.107.159228
3. Agarwal AK, Garg A. Genetic basis of lipodystrophies and management of metabolic complications. Annu Rev Med 2006; 57:297-311; PMID:16409151; http://dx.doi.org/10.1146/annurev.med.57.022605.114424
4. Liu J, Fox CS, Hickson DA, May WD, Hairston KG, Carr JJ, Taylor HA. Impact of abdominal visceral and subcutaneous adipose tissue on cardiometabolic risk factors: the Jackson Heart Study. J Clin Endocrinol Metab 2010; 95:5419-26; PMID:20843952; http://dx.doi.org/10.1210/jc.2010-1378
5. Esser N, Legrand-Poels S, Piette J, Scheen AJ, Paquot N. Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. Diabetes Res Clin Pract 2014; 13;In press; PMID:24798950
6. Misra A, Peethambaram A, Garg A. Clinical features and metabolic and autoimmune derangements in acquired partial lipodystrophy: report of 35 cases and review of the literature. Medicine (Baltimore) 2004; 83:18-34; PMID:14747765; http://dx.doi.org/10.1097/01.md.0000111061.69212.59
7. Rodeheffer MS, Birsoy K, Friedman JM. Identification of white adipocyte progenitor cells in vivo. Cell 2008; 135:240-9; PMID:18835024; http://dx.doi.org/10.1016/j.cell.2008.09.036
8. Lee YH, Petkova AP, Mottillo EP, Granneman JG. In vivo identification of bipotential adipocyte progenitors recruited by β3-adrenoceptor activation and high-fat feeding. Cell Metab 2012; 15:480-91; PMID:22482730; http://dx.doi.org/10.1016/j.cmet.2012.03.009
9. Berry R, Rodeheffer MS. Characterization of the adipocyte cellular lineage in vivo. Nat Cell Biol 2013; 15:302-8; PMID:23434825; http://dx.doi.org/10.1038/ncb2696
10. Magnuson MA, Osipovich AB. Pancreas-specific Cre driver lines and considerations for their prudent use. Cell Metab 2013; 18:9-20; PMID:23823474; http://dx.doi.org/10.1016/j.cmet.2013.06.011
11. Schmidt-Supprian M, Rajewsky K. Vagaries of conditional gene targeting. Nat Immunol 2007; 8:665-8; PMID:17579640; http://dx.doi.org/10.1038/ni0707-665
12. Kang S, Kong X, Rosen ED. Adipocyte-specific transgenic and knockout models. Methods Enzymol 2014; 537:1-16; PMID:24480338; http://dx.doi.org/10.1016/B978-0-12-411619-1.00001-X
13. Graves RA, Tontonoz P, Platt KA, Ross SR, Spiegelman BM. Identification of a fat cell enhancer: analysis of requirements for adipose tissue-specific gene expression. J Cell Biochem 1992; 49:219-24; PMID:1644859; http://dx.doi.org/10.1002/jcb.240490303
14. Abel ED, Peroni O, Kim JK, Kim YB, Boss O, Hadro E, Minnemann T, Shulman GI, Kahn BB. Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. Nature 2001; 409:729-33; PMID:11217863; http://dx.doi.org/10.1038/35055575
15. He W, Barak Y, Hevener A, Olson P, Liao D, Le J, Nelson M, Ong E, Olefsky JM, Evans RM. Adipose-specific peroxisome proliferator-activated receptor gamma knockout causes insulin resistance in fat and liver but not in muscle. Proc Natl Acad Sci U S A 2003; 100:15712-7; PMID:14660788; http://dx.doi.org/10.1073/pnas.2536828100
16. Mullican SE, Tomaru T, Gaddis CA, Peed LC, Sundaram A, Lazar MA. A novel adipose-specific gene deletion model demonstrates potential pitfalls of existing methods. Mol Endocrinol 2013; 27:127-34; PMID:23192980; http://dx.doi.org/10.1210/me.2012-1267
17. Urs S, Harrington A, Liaw L, Small D. Selective expression of an aP2/Fatty Acid Binding Protein 4-Cre transgene in non-adipogenic tissues during embryonic development. Transgenic Res 2006; 15:647-53; PMID:16952017; http://dx.doi.org/10.1007/s11248-006-9000-z
18. Lee KY, Russell SJ, Ussar S, Boucher J, Vernochet C, Mori MA, Smyth G, Rourk M, Cederquist C, Rosen ED, et al. Lessons on conditional gene targeting in mouse adipose tissue. Diabetes 2013; 62:864-74; PMID:23321074; http://dx.doi.org/10.2337/db12-1089
19. Shan T, Liu W, Kuang S. Fatty acid binding protein 4 expression marks a population of adipocyte progenitors in white and brown adipose tissues. FASEB J 2013; 27:277-87; PMID:23047894; http://dx.doi.org/10.1096/fj.12-211516
20. Eguchi J, Wang X, Yu S, Kershaw EE, Chiu PC, Dushay J, Estall JL, Klein U, Maratos-Flier E, Rosen ED. Transcriptional control of adipose lipid handling by IRF4. Cell Metab 2011; 13:249-59; PMID:21356515; http://dx.doi.org/10.1016/j.cmet.2011.02.005
21. Wang ZV, Deng Y, Wang QA, Sun K, Scherer PE. Identification and characterization of a promoter cassette conferring adipocyte-specific gene expression. Endocrinology 2010; 151:2933-9; PMID:20363877; http://dx.doi.org/10.1210/en.2010-0136
22. Muzumdar MD, Tasic B, Miyamichi K, Li L, Luo L. A global double-fluorescent Cre reporter mouse. Genesis 2007; 45:593-605; PMID:17868096; http://dx.doi.org/10.1002/dvg.20335
23. Rivers LE, Young KM, Rizzi M, Jamen F, Psachoulia K, Wade A, Kessaris N, Richardson WD. PDGFRA/NG2 glia generate myelinating oligodendrocytes and piriform projection neurons in adult mice. Nat Neurosci 2008; 11:1392-401; PMID:18849983; http://dx.doi.org/10.1038/nn.2220
24. Kang SH, Fukaya M, Yang JK, Rothstein JD, Bergles DE. NG2+ CNS glial progenitors remain committed to the oligodendrocyte lineage in postnatal life and following neurodegeneration. Neuron 2010; 68:668-81; PMID:21092857; http://dx.doi.org/10.1016/j.neuron.2010.09.009
25. Driskell RR, Lichtenberger BM, Hoste E, Kretzschmar K, Simons BD, Charalambous M, Ferron SR, Herault Y, Pavlovic G, Ferguson-Smith AC, et al. Distinct fibroblast lineages determine dermal architecture in skin development and repair. Nature 2013; 504:277-81; PMID:24336287; http://dx.doi.org/10.1038/nature12783
26. Festa E, Fretz J, Berry R, Schmidt B, Rodeheffer M, Horowitz M, Horsley V. Adipocyte lineage cells contribute to the skin stem cell niche to drive hair cycling. Cell 2011; 146:761-71; PMID:21884937; http://dx.doi.org/10.1016/j.cell.2011.07.019
27. Roesch K, Jadhav AP, Trimarchi JM, Stadler MB, Roska B, Sun BB, Cepko CL. The transcriptome of retinal Müller glial cells. J Comp Neurol 2008; 509:225-38; PMID:18465787; http://dx.doi.org/10.1002/cne.21730
28. Orr-Urtreger A, Bedford MT, Do MS, Eisenbach L, Lonai P. Developmental expression of the alpha receptor for platelet-derived growth factor, which is deleted in the embryonic lethal Patch mutation. Development 1992; 115:289-303; PMID:1322271
29. Orr-Urtreger A, Lonai P. Platelet-derived growth factor-A and its receptor are expressed in separate, but adjacent cell layers of the mouse embryo. Development 1992; 115:1045-58; PMID:1451656
30. Hamilton TG, Klinghoffer RA, Corrin PD, Soriano P. Evolutionary divergence of platelet-derived growth factor alpha receptor signaling mechanisms. Mol Cell Biol 2003; 23:4013-25; PMID:12748302; http://dx.doi.org/10.1128/MCB.23.11.4013-4025.2003
31. Pringle NP, Richardson WD. A singularity of PDGF alpha-receptor expression in the dorsoventral axis of the neural tube may define the origin of the oligodendrocyte lineage. Development 1993; 117:525-33; PMID:8330523
32. Seale P, Bjork B, Yang W, Kajimura S, Chin S, Kuang S, Scimè A, Devarakonda S, Conroe HM, Erdjument-Bromage H, et al. PRDM16 controls a brown fat/skeletal muscle switch. Nature 2008; 454:961-7; PMID:18719582; http://dx.doi.org/10.1038/nature07182
33. Sanchez-Gurmaches J, Hung CM, Sparks CA, Tang Y, Li H, Guertin DA. PTEN loss in the Myf5 lineage redistributes body fat and reveals subsets of white adipocytes that arise from Myf5 precursors. Cell Metab 2012; 16:348-62; PMID:22940198; http://dx.doi.org/10.1016/j.cmet.2012.08.003
34. Lepper C, Fan CM. Inducible lineage tracing of Pax7-descendant cells reveals embryonic origin of adult satellite cells. Genesis 2010; 48:424-36; PMID:20641127; http://dx.doi.org/10.1002/dvg.20630
35. Rosenwald M, Perdikari A, Rülicke T, Wolfrum C. Bi-directional interconversion of brite and white adipocytes. Nat Cell Biol 2013; 15:659-67; PMID:23624403; http://dx.doi.org/10.1038/ncb2740
36. Berry R, Church CD, Gericke MT, Jeffery E, Colman L, Rodeheffer MS. Imaging of adipose tissue. Methods Enzymol 2014; 537:47-73; PMID:24480341; http://dx.doi.org/10.1016/B978-0-12-411619-1.00004-5
37. Church CD, Berry R, Rodeheffer MS. Isolation and study of adipocyte precursors. Methods Enzymol 2014; 537:31-46; PMID:24480340; http://dx.doi.org/10.1016/B978-0-12-411619-1.00003-3