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Reprogramming mature terminally differentiated adipocytes to induced pluripotent stem cells

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Abstract Mature adipocytes are terminally differentiated somatic cells. Here, we report the successful generation of induced pluripotent stem (iPS) cells from mouse mature adipocytes by forced expression of six transcription factors (Oct4, Sox2, c-Myc, Klf4, Rarγ, and Lrh1) with a piggyBac transposon-based strategy. The resulting iPS cells were pluripotent as evidenced by the fact that they stained positive for alkaline phosphatase, expressed high levels of key pluripotency markers including Oct4, Nanog, and SSEA1, and remained pluripotent on a 2i media. In vitro differentiation of the iPS cells showed that the cell derivatives of all three germ layers could be readily obtained through formation of embryoid bodies. Most importantly, these adipocyte-derived iPS cells were capable of producing chimera with high frequencies when reintroduced into early-stage embryos and transmitted through the germ line. This study demonstrates that the new six-factor reprogramming technology facilitates the reset of the terminally differentiated adipocytes to the ground state of pluripotency, enabling us to fully explore the potential of mature adipocytes as a viable cell source for regenerative medicine.

Keywords Adipocyte · Induced pluripotent stem cell · PiggyBac

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SPECIAL TOPIC: Stem cell, Basis and Application

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1 Introduction

In 2006, Shinya Yamanaka in Kyoto University launched the field of induced pluripotent stem (iPS) cells by demonstrating that mouse somatic fibroblasts can be reprogrammed to undifferentiated and pluripotent stem cells through ectopic expression of defined factors [1]. Later on, the successful reprogramming of human iPS cells from skin fibroblast has been reported [2]. iPS technology has attracted substantial interests and revolutionized the regenerative medicine since it allows the generation of patient-specific and disease-specific pluripotent cells and is ethically less problematic than the human therapeutic cloning methodology.

With the fast progress in this field, several strategies for delivering reprogramming factors have been proved successful. Except for the conventional retrovirus and lentivirus, non-integrating adenovirus [3], virus-free plasmid transfection [4, 5], self-replicating and selectable episomes [6], and excisable transposons [7, 8] have also been

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developed. Among them, piggyBac transposition-based reprogramming approach surpasses most of the existing methods by achieving the "traceless" deletion of the transgenes while retaining the high reprogramming efficiency (0.1 %–1 %) comparable to that of the integrating virus.

At the same time, several reprogramming factors have been reported that can either replace or enhance the efficacy of the classical "Yamanaka factors". Human somatic cells can be reprogrammed to pluripotent stem cells with Oct4, Sox2 Nanog, and Lin28 [9]. In addition, the nuclear receptors Esrrb and Nr5a2 were shown to be a reprogramming factor capable of replacing Klf4 and Oct4, respectively, in the reprogramming of mouse embryonic fibroblasts (MEFs) [10, 11]. Most recently, Wang et al. [12] demonstrated rapid reprogramming of both human and mouse fibroblasts with high efficiency and quality using the classical Yamanaka factors plus two nuclear hormone receptors Rarγ and Lrh1. The inclusion of the two nuclear hormone receptors shortens the reprogramming process from 3 to 4 weeks to 4 to 5 days.

To date, many cell types of mouse and human somatic stem cells have been reprogrammed to pluripotency, including hematopoietic stem cells, adipocytes stem cells, and neural stem cells [13, 14]. For instance, neural stem cells could be reprogrammed to iPS using less than four Yamanaka factors, possibly due to the fact that these adult stem cells possess some "stemness" or epigenetically favorable state conducible to be induced back to pluripotency. Interestingly, terminally differentiated mesoderm-derived cells, such as mature B and circulating T lymphocytes, were reprogrammed to iPS cells [15, 16], breaking the original notion that differentiated cells are refractory to reprogramming. In case of the endoderm-derived cells, pancreatic β cell and hepatocytes were also successfully reprogrammed into iPS cells, albeit at very low efficiencies [17, 18]. However, one of the practical questions in iPS cell-based autologous or disease-specific regenerative medicine is the uneasy access of starting cells. Therefore, their potential application in regenerative medicine is limited by the difficulty of obtaining the source cells.

White adipocytes are mature, fully differentiated somatic cells that serve as a lipid-storing site in the body. It is characterized by a single large lipid droplet and small nucleus pushed aside at the border of the cell. An average adult is estimated to have 30 billion fat cells with a weight of 30 lbs. According to the American Society for Aesthetic Plastic Surgery, liposuction is the most common plastic surgery procedure in the USA. Therefore, unlike most other cell types or tissues, biopsies of fat tissue can be obtained in this relatively safe and routine way. Although it was demonstrated that adult human and mouse adipose-derived stem cells can be reprogrammed to iPS cells with substantially higher efficiencies than those reported for human and mouse fibroblasts [19], the reprogramming of mature white adipocytes has not been reported.

Here, we reported the generation of iPS cells from the terminally differentiated mouse adipocytes by using the recently reported "six transcription factors", i.e., the classical "Yamanaka factors" plus Rarγ and Lrh1 in a piggyBac transposon-based delivery system. These reprogrammed iPS cells are fully pluripotent evidenced by the expression of pluripotency markers, ability to contribute to cell types of all germ layers, generation of chimeric mice, and transmission through the germ line. Our results thus provide genetic proof that terminally differentiated cells, even as inert adipocytes, can be reprogrammed to pluripotent cells, implying that in vitro reprogramming is not restricted to certain cell types or differentiation stages. It is equally noteworthy that the "six-factor" transposition approach might represent a more efficient way to reprogram other terminally differentiated cells resistant to reprogramming using the classical Yamanaka factors.

2 Materials and methods

2.1 Mice

The aP2-Cre mice were purchased from the Jackson Laboratory. $Appl2^{flox/flox}$ mice were generated in house by standard gene manipulation protocol. Both mice were in C57BL/6J×129Sv background. The aP2-Cre mice were crossed with $Appl2^{flox/flox}$ mice to obtain aP2-Cre/$Appl2^{flox/flox}$ mice. Genotyping was performed by PCR with the following primer pairs: aP2-Cre Forward: 5′-AGCGATGGATTTCC GTCTCTGG-3′; aP2-Cre Reverse: 5′-AGCTTGCATGAT CTCCGGTATTGAA-3′; $Appl2^{flox/flox}$ Forward: 5′-GCA TCGCATTGTCTGAGTAGGTG-3′; $Appl2^{flox/flox}$ Reverse: 5′-CCTCCCTCTGTTGAACCAGGAACG-3′.

2.2 Isolation of mature adipocytes

Isolation of mature adipocytes from fat tissue was performed following a previously described method with minor modifications [20]. Briefly, approximately 1 g of mouse subcutaneous fat tissue was freshly dissected out, minced, and digested in 0.1 % (w/v) type I collagenase (Weijia, Guangzhou, China) at 37 °C for 1 h with gentle agitation. After filtration and centrifugation at 135 *g* for 3 min, the floating top layer containing mature adipocytes was collected. The mature adipocytes are readily distinguishable from the precursor cells since their buoyant density is less than buffer, and thus, they were cultured by