Stem Cell Research & Therapeutics
SCRT, 4(1): 142-145
www.scitcentral.com

ISSN: 2474-4646

Review Article: Open Access

Critical Role of TGFβ Pathway in the Differentiation of Human Induced Pluripotent Stem Cells into Fat-Burning Adipocytes

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Received May 23, 2019; Accepted June 05, 2019; Published November 13, 2019

ABSTRACT

Alternative strategies are urgently required to fight obesity and associated metabolic disorders including diabetes and cardiovascular diseases. Brown and brown-like beige adipocytes (BAs) store fat, but in contrast to white adipocytes, they are equipped to dissipate energy stored. Therefore, BAs represent promising cell targets to counteract obesity. However, the scarcity of BAs in adults is a major limitation for a BA-based therapy of obesity, and the notion to increase the BA mass by transplanting BA progenitors (BAPs) in obese patients recently emerged. The capacity of human induced pluripotent stem cells (hiPSCs) to generate BAPs at a high efficiency offers the opportunity to produce an unlimited number of patient-matched BAs. However, hiPSC-BAPs display a low adipogenic capacity that hampered their use both in cell-based therapy and basic research. Recently we, and others, have identified the critical role of TGFβ pathway in switching off differentiation of hiPSC-BAPs in classical 2D culture and also in a 3D beige adiposphere model better mimicking adipocytes *in vivo*. Inhibition of TGFβ pathway unlocks differentiation of hiPSC-BAPs making this cell model a suitable tool for therapeutic transplantation. In contrast to BAPs derived from human iPSCs, inhibition of TGF β pathway is not a requisite for differentiation of preadipocytes derived from adult adipose tissues. This observation suggests that hiPSC-BAPs and adult adipose tissue-preadipocytes are at different stages of the adipose progenitor hierarchy.

Keywords: TGFβ pathway, Human induced pluripotent stem cells, Beige adipocytes, Adipocyte progenitors, Stem cell-based therapy, Obesity

Abbreviations: TGF: Transforming Growth Factor; BA: Brown and Beige Adipocyte; BAP: Brown and Beige Adipocyte Progenitor; hiPSC: Human Induced Pluripotent Stem Cell

INTRODUCTION

Obesity and associated metabolic disorders such as diabetes and cardiovascular diseases are major health problems. Obesity results from an imbalance between calorie intake and energy expenditure. In mammals, three types of adipocytes coexist, i.e. brown, beige and white, which are all involved in energy balance regulation while having opposite functions. White adipocytes are involved in energy storage and their accumulation marks obesity. Therapies based to reduce energy intake are difficult to follow in our modern life, and current anti-obesity drugs cause important side effects for the patients that limits their use. Bariatric surgery has proven efficiency for obesity, although long-term complications and obesity relapse may appear. Therefore, alternative strategies to increase energy expenditure with the identification of new anti-obesity targets are urgently required. In contrast to white adipocytes, classical brown adipocytes and brown-like adipocytes (BAs), also named beige or brite adipocytes because dispersed in white adipose tissues, are specialized in energy expenditure. Upon activation, BAs consume metabolic substrates and burn fat via the mitochondrial uncoupling protein-1 (UCP-1). Moreover, the ability of BAs to actively drain circulating glucose and triglycerides to oxidize them can prevent hyperglycemia and hypertriglyceridemia. Therefore, BAs represent promising cell targets to counteract obesity in human. However, there is a major limitation for a BA-based treatment of obesity that is that BAs hold a minor fraction of adipose tissue in human and disappear from most areas with age, persisting only around deeper organs. In addition, BA activity is lower in overweight and obese individuals than in

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Citation: Yao X & Dani C. (2019) Critical Role of TGFβ Pathway in the Differentiation of Human Induced Pluripotent Stem Cells into Fat-Burning Adipocytes. Stem Cell Res Th, 4(1): 142-145.

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leans [1,2]. Hence, the notion to increase the BA mass by transplanting BA progenitors (BAPs) in obese patients as a therapeutic alternative to counteract obesity and its associated metabolic complications recently emerged. The proof-of-concept has been validated in murine models as it has been reported that implants of mouse BAT or of human BAs purified from capillary networks were able to restore normoglycemia in diabetic mice and to reduce obesity in Ob/Ob mice [3-6]. It is interesting to note that small amount of transplants was sufficient to display a beneficial effect, reflecting that in addition to acting as a glucose and energy sink; BAs secrete adipokines that could also contribute to metabolic effects [7]. Therefore, a reliable source of human BAs was urgently needed and induced pluripotent stem cells (iPSCs) appear as a suitable source of BAPs having a high adipogenic capacity only when the TGFβ pathway is switch off.

THE TGF β PATHWAY GOVERNS THE DIFFERENTIATION OF hiPSCs INTO BAs

Induced pluripotent stem cells (iPSCs) represent an abundant source of multiple cell types of therapeutic interest for drug screening as well as for transplantation [8,9]. Nakao's group was the first to demonstrate the capacity of hiPSCs to generate white adipocytes [10]. Total differentiated hiPSC populations, but not purified adipose progenitors, were transplanted into mice. Indeed, differentiated hiPSC cultures can be enriched with adipocytes, but still contain other cell types that are unsuitable for transplantation, including undifferentiated hiPSCs that can form teratomas. An alternative to eliminate hiPSC capacity to form teratomas consists in purifying progenitors of interest during hiPSC differentiation. Ahfeldt et al. [11] and Mohsen-Kanson et al. [12] were able to generate pure BAs from hiPSCs that displayed a high adipogenic capacity but only following transduction with adipogenesis master genes. The need to genetically modify hiPSCs-derived progenitors to generate adipocytes clearly illustrates the low adipogenic potential of hiPSCs. This feature represented a bottleneck hampering their clinical use [13].

Several factors, such as ascorbic acid, EGF and hydrocortisone have been shown to regulate hiPSC-BAPs differentiation [12,14,15]. However, TGFβ signaling holds a pivotal role. Members of the TGFβ family are expressed in various tissues where they have been shown to regulate various biological processes including regulation of apoptosis, proliferation and differentiation of different cell types [16]. The TGFβ pathway emerged as a critical anti-adipogenic player through the activation of Smad 2/3 [17-19]. Deletion of TGFβ receptor 1 in mice has been shown to promote beige adipogenesis within white adipose tissue, supporting a model where TGFβ receptor signalling play a role in regulating the pool of beige adipose progenitors [20]. It has been shown that Smad2/3 pathway was active during hiPSC-BAP differentiation suggesting that bioactive TGFβ family members were secreted that might lock differentiation [14]. In agreement with this hypothesis, Su et al. [21] showed more recently that expression of TGFβ-ligands and receptors increased from the differentiation of FOXF1 mesoderm progenitors towards adipocytes during *in vitro* development of hiPSCs [21]. Then, the anti adipogenic role of the TGFβ pathway has been functionally demonstrated thanks to the use of the TGFβ inhibitor SB431542 [22]. Inhibition of active Smad 2/3 pathway upon SB431542 addition during hiPSC-BAP differentiation induced a dramatically increased of UCP1 expression and of the number of mature beige adipocytes [14,15,21,23]. In addition, inhibition of TGFβ signaling in hiPSC-mesenchymal stem cells, i.e., before induction of adipogenic differentiation, promoted the generation of adipocytes [21]. Altogether, these data underline the critical role of TGFβ pathway in the commitment of hiPSC into the adipogenic lineage. They indicate that TGFβ signalling inhibition enhances the conversion of mesenchymal stem cells into adipogenic progenitors and switches on the differentiation of progenitors into mature beige adipocytes.

INHIBITION OF TGFβ PATHWAY IS REQUIRED ONLY DURING THE FIRST DAYS OF DIFFERENTIATION OF hiPSC-3D BEIGE ADIPOSPHERES

Inhibition of TGFβ pathway is required to induce differentiation of hiPSC-derived adipose progenitor cells into adipocytes, whereas is not for the differentiation of progenitors derived from human adult adipose tissues. The low hiPSC-BAP adipogenic capacity compared to adult-BAPs is reminiscent of an observation reported by Han et al. [24]. These authors observed that epididymal adipose tissue, which undergoes early development in mouse, is composed of progenitor cells that lack their adipogenic capacity once isolated from the tissue. In contrast to cells derived from other fat pads that developed later, epididymal fat cells required a 3D structure and a different micro-environment to undergo differentiation. Therefore, among the reasons to explain the weak efficacy of hiPSC-BAP differentiation, one can mention the culture conditions that do not mimic the phenotype of the cells and their physiological microenvironment within the adipose tissue. Cells are classically grown as monolayer, which poorly reflects the *in vivo* situation [25]. In contrast, the cell-cell and cell-extracellular matrix interactions are promoted in 3D configurations. Therefore, 3D cultures represent a bridge between traditional cell culture and live tissue. HiPSC-BAPs can form 3D spheroids able to differentiate into beige adipospheres expressing UCP-1 (**Figure 1B**, Yao X and Dani C, unpublished data). In fact, beige adipospheres revealed a TGFβ pathway depend phase only during the first days of spheroid differentiation.

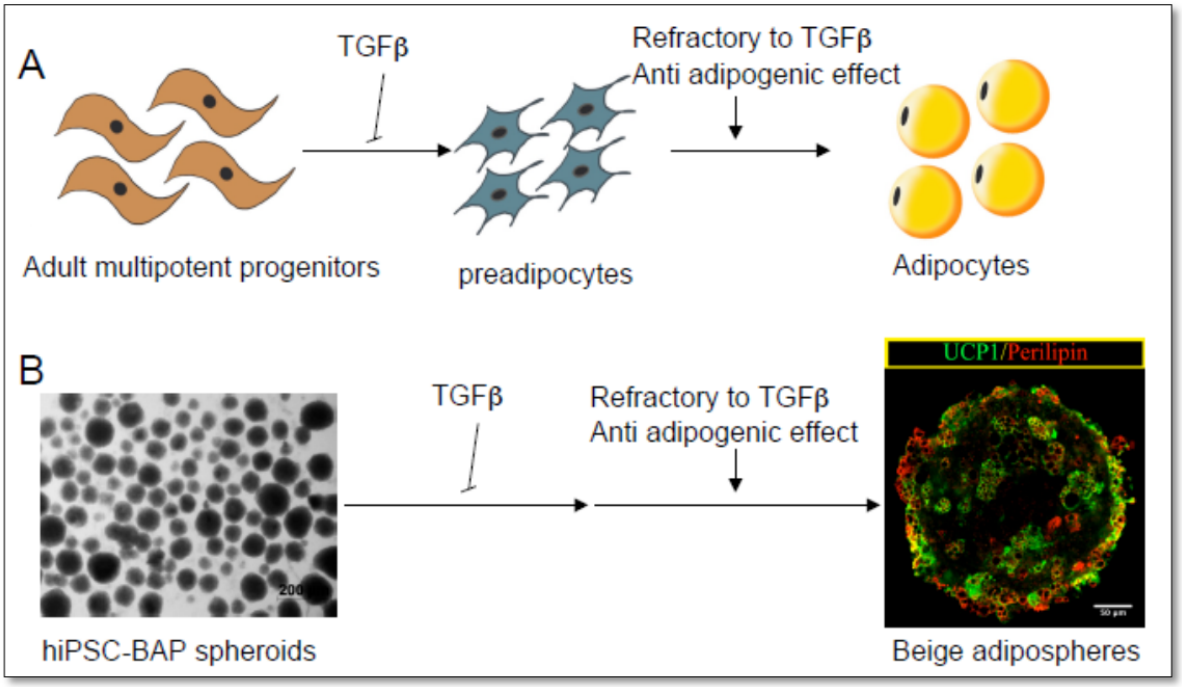

Figure 1. Commitment towards the adipogenic lineage of adult adipose tissues-and of hiPSC-progenitors requires inhibition of TGFβ signalling pathway. A) Multipotent progenitors at the stem of mesenchymal cell hierarchy of adipocyte formation require TGFβ inhibition only for their differentiation into preadipocytes (adapted from [26]). B) Beige progenitors derived from hiPSCs can form spheroids and undergo differentiation into adipospheres expressing UCP1. Only the first days of spheroid differentiation requires TGFβ inhibition.

CONCLUSION

Interestingly, Seale group has recently proposed a mesenchymal progenitor cell hierarchy in adipose tissue where the multipotent progenitor cell required inhibition of TGFβ pathway for its differentiation into preadipocytes. Then, preadipocytes are refractory to the anti adipogenic action of TGFβ to differentiate into adipocytes (**Figure 1A**, [26]). As discussed above, differentiation of hiPSC-BAP spheroids display also a sensitive and a refractory phase to anti adipgenic effect of TGFβ. It is tempting to speculate that BAPs derived from hiPSCs resemble the multipotent progenitor subpopulation in adult adipose tissue at the origin of preadipocytes. Further analyses are required to test this hypothesis. Numerous other issues have also to be solved before a therapeutic use of iPSCs in the obesity field, but identification of pathways governing the differentiation of BAPs at a high level as well as their capacity to form 3D adipospheres open the opportunity of using hiPSCs advantages for anti-obesity therapy.

ACKNOWLEDGEMENT

The work has been supported by the ANR-18-CE18-0006-01.

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