

Chapter 2

Adipocyte Progenitors from Human Pluripotent Stem Cells

A.-L. Hafner and C. Dani

Abstract The current epidemic of obesity and overweight has caused a surge of interest in the study of adipose tissue formation. Much progress has been made in defining the transcriptional networks controlling the terminal differentiation of preadipocytes into mature adipocytes. However, the early steps that direct pluripotent stem cells down the adipocyte lineage remain largely unknown. Similarly, the study of the developmental origins of adipocytes has been largely overlooked until now. Induced Pluripotent Stem Cells (iPSCs) provide a novel cell model for investigating human adipocyte ontogenesis. From a clinical standpoint, many issues have to be resolved before using hiPS-derived adipocyte progenitors in cell-based therapeutic for obesity. However, the differentiation of iPS cells towards the adipogenic lineage offers a unique opportunity to purify brown adipocytes from patients, which could lead to the development of autologous transplantation procedures to counteract obesity.

This chapter summarizes recent work on the developmental origins and the therapeutic potential of adipocyte progenitors from human pluripotent stem cells.

Keywords Adipocyte progenitors • Pluripotent stem cells • Adipose tissue • Adipocyte development

2.1 Introduction

In mammals, three types of adipocytes coexist, i.e. brown, beige/brite and white adipocytes, which are all involved in regulation of the energy balance while having opposite functions. White adipose tissue (WAT) is dispersed throughout the body and is mainly involved in energy storage. In contrast to WAT, brown adipose tissue (BAT) is specialized in energy expenditure. Activated BAT consumes metabolic substrate and burns fat to produce heat thanks to the Uncoupling Protein (UCP)1. Beige/brite adipocytes have been recently described as brown-like adipocytes and represent a third type

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of adipocytes that are in WAT (Wu et al. 2012). Activation of BAT or recruitment of beige/brite adipocytes leads to powerful anti obesity and anti-diabetic effects in mice. Independent laboratories have reported that adult BAT transplantation could reverse metabolic disorders in mice, making brown/brite adipocytes transplantation a credible approach to treat obesity and type 2 diabetes (Stanford et al. 2013; Gunawardana and Piston 2010; Liu et al. 2015). More recently, it has been shown that beige/brite adipocytes derived from the capillaries of human subcutaneous adipose tissue were able to enhance glucose tolerance after implantation into mice (Min et al. 2016). Therefore, the notion of transplanting brown or beige/brite adipocyte progenitors (BAPs) in obese patients as a therapeutic approach to counteract obesity and its associated metabolic complications has recently emerged. However, BAT represents a minor fraction of adipose tissue in humans and disappears from most areas with age, persisting only around deeper organs (Frontini and Cinti 2010). Human BAPs are hard to isolate in this regard. Therefore, a cellular source is urgently needed to amplify and characterize human BAPs. Pluripotent stem cells appeared recently as a powerful model to decipher human adipocyte ontogenesis and as an unlimited source of autologous BAPs for transplantation.

2.2 Potential of Human PSCs to Generate Adipocytes

Following the pioneer work of Yamanaka's group on the generation of patient-specific induced pluripotent stem cells (hiPSCs) by reprogramming fibroblasts (Takahashi et al. 2007), hiPSCs emerged as an unlimited source of APs for autologous cell-based therapy to treat obesity. The capacity of hiPSCs to generate functional white adipocytes was first reported by Nakao's group. Interestingly, adipocytes generated from hiPSCs can maintain their functional properties for several weeks after transplantation into nude mice (Taura et al. 2009; Noguchi et al. 2013). These data revealed that hiPSC-adipocytes could potentially be used to correct metabolic parameters in lipodystrophic patients. In these experiments, differentiated hiPSCs, but not purified APs, were transplanted into mice. Indeed, differentiated hiPSC cultures are enriched with adipocytes after adipogenic induction, but also contain several other cell types that are undesirable for transplantation, including undifferentiated iPSCs that can form teratomas. This indicates that purification of hiPSC-APs with a high adipogenic capacity is required prior to proposing an hiPSC-based therapeutic approach. Nishio et al. (2012) developed a procedure to generate functional brown adipocytes at a high frequency from hiPSCs using a hematopoietic cocktail to induce hiPSC differentiation. Remarkably, hiPSC-brown adipocytes were able to improve glucose tolerance after transplantation in mice. This report indicated that hiPSCs could potentially be used to generate brown adipocytes with therapeutic properties. Finally, Ahfeldt et al. (2012) were able to generate pure brown and white adipocytes from hiPSCs, but only following transduction with master genes of adipogenesis. The need to genetically modify hiPSC-APs to generate adipocytes clearly illustrates the low adipogenic potential of hiPSC-derived APs.

2.3 Adipogenic Capacity of hiPSC-Derived Adipocyte Progenitors

Chen et al. first underlined the limited capacity of hiPSC-derived mesenchymal cells to undergo adipocyte differentiation, a feature that has often been observed but not always highlighted (Chen et al. 2012). The reasons of this feature are unknown and hamper their use both in cell-based therapy and basic research. Interestingly, some authors claim that the low differentiation capacity is limited to adipogenesis since hiPSC-derived mesenchymal cells are able to differentiate towards chondrogenic and osteogenic lineages at high levels (Xu et al. 2004; Boyd et al. 2009; Karlsson et al. 2009). The low adipogenic capacity of hiPSC-derived APs is unlikely to be due to the derivation method. In fact, there are two main approaches to differentiate pluripotent stem cells into adipocyte progenitors. One strategy involves embryoid body (EB) formation. In this approach, suspension cultures allow pluripotent stem cells to form 3-dimensional structures called EB. This step, models the *in vitro* embryonic development with the commitment of cells into the three primary germ layers. EBs are then seeded onto culture dishes and, after a proliferation step, outgrowth cells are maintained in a mesenchymal culture medium. Subsequently, adherent cells display a fibroblast-like morphology and acquire specific mesenchymal markers after serial passages (Brown et al. 2009; Lee et al. 2010). An alternative strategy involves direct differentiation of pluripotent stem cells without the EB step (Hynes et al. 2013). Another version of this protocol relies on the spontaneous differentiation of pluripotent stem cells into mesenchymal cells (Diederichs and Tuan 2014). The low adipogenic potential was observed in these reports. Finally, mesenchymal cells derived from different hiPSC lines generated from different donors, or from the same hiPSC clone using different derivation approaches, have the same adipogenic features (Hynes et al. 2013; Diederichs and Tuan 2014). Interestingly, the low differentiation potential is not restricted to hiPSC since mesenchymal cells derived from human embryonic stem cells (hESCs) display the same feature, thus ruling out the possibility that the low hiPSC-AP adipogenic capacity could be due to the reprogramming process or to an epigenetic mechanism. Several hypotheses could be put forward to explain the low adipogenic capacity of hiPSC-APs compared to adult adipocyte progenitors. We favoured the hypothesis that the range of factors or culture conditions required to induce adipocyte differentiation of adipocyte progenitors derived from adult tissues and from embryonic-like cells could differ (Hafner and Dani 2014). The low hiPS-AP adipogenic capacity is reminiscent of an earlier observation reported by Han and colleagues (2011). The authors observed that epididymal adipose tissue, which undergoes postnatal development in mouse, is composed of multipotent progenitor cells but lack an adipogenic capacity *in vitro*. In contrast to cells derived from other fat pads, epididymal fat cells require three-dimensional culture conditions and a different micro-environment to undergo differentiation. These results emphasise that the micro-environment has a critical role in differentiation but could differ between adult and embryonic-like cells. Recently, Mohsen-Kanson et al. have selectively generated white and brown

adipocyte progenitors from hiPSCs (Mohsen-Kanson et al. 2014). Then, they have identified a set of factors, including angiogenic factors, capable of governing hiPSC-BAP differentiation at a level similar to that of BAPs derived human adult adipose tissue (Hafner et al. 2016). Altogether, these data highlighted that a specific micro-environment is requirement to switch on hiPSC-brown adipogenesis and opens new opportunities to develop alternative strategies to counteract obesity.

2.4 Developmental Origins of Human Brown Adipocyte Progenitors

A better knowledge of the developmental origins of the different adipose progenitors is central to understand the diversity of adipose tissues. The emerging picture arising from lineage tracing tools is that brown, beige/brite and white adipocytes are generated from cells having multiple origins. It has been first proposed that classical brown and not beige/brite adipocytes, originate from a precursor expressing myogenic transcription factor-5 (Myf-5) (Seale et al. 2008). However, other studies revealed that all colours of adipocytes also originate from either Myf5-positive or negative progenitors (Sanchez-Gurmaches et al. 2012). It has been also further proposed that a subpopulation of beige/brite adipocytes derives from smooth muscle cells (Long et al. 2014). Recently, Sanchez-Gurmaches et al. have been nicely reviewed the complexity of adipocyte origins (Sanchez-Gurmaches et al. 2016). We have also demonstrated that white adipocytes have two embryonic origins, i.e. neuroectoderm and mesoderm, depending on their body localization in rodents (Billon and Dani 2011). APs derive from mesenchymal stem cells, which themselves are thought to arise from mesoderm only. However, it is worth noting that during development of higher vertebrates, the mesoderm is not the only germ layer source of mesenchymal cells. In the head, for instance, the facial bones have been shown to derive from the neural crest (NC). The NC is a vertebrate cell population that arises from the neuroectoderm. After neural tube closure, NC cells undergo an epithelio-mesenchyme transition and migrate to diverse regions in the developing embryo, where they differentiate into various cell types. In the head and neck, the NC also yields mesenchymal precursors differentiating into connective tissue cells (reviewed in Dupin et al. 2006). Adipogenesis of mouse Embryonic Stem (mES) cells *in vitro* provided the first model to investigate the earliest steps of adipocyte development and revealed the surprising conclusions regarding the ontogeny of such cells in the NC, better known to generate neuronal cells and glial cells.

In the mouse ES cell system, the generation of adipocytes is dependent on an early and transient exposure of EBs to retinoic acid (RA) and a subsequent treatment with conventional adipogenic factors. In a first attempt to unravel the events underlying the formation of mesenchymal derivatives in RA-treated mES cells, Kawaguchi et al. examined the expression of various mesodermal and mesenchymal markers in early EBs. Surprisingly, they noticed that treatment with RA resulted in

a sharp reduction in several mesodermal markers, as well as in the suppression cardiomyocyte formation, suggesting that RA reduces overall mesoderm formation in mESCs (Kawaguchi et al. 2005). They showed that *sox9*, *sox10*, *foxD3*, and *runx2*, which all play an important role in NC formation and/or mesenchymal condensation, were upregulated upon RA-treatment. Together, these data suggest that neuroectoderm/NC is the major source of mesenchymal cells in RA-treated mESCs. To test this hypothesis with respect to adipocytes, we checked whether adipocytes could be obtained from NCCs using a lineage tracing approach in mouse. This study revealed adipocytes derived from NC in craniofacial adipose depots. In contrast, no NC-derived adipocytes could be detected in truncal adipose depots, including subcutaneous, perirenal, periepididymal, and interscapular tissues. These data therefore provide new information about the ontogeny of the adipocyte lineage and demonstrate that during normal development, a subset of adipocytes in the craniofacial region originates from NC, and not from mesoderm (Billon et al. 2007). However, the white or brown phenotype of adipocytes derived from the cranial neural crest was not investigated at that time (see below). The role of RA in the early steps of adipocyte development remains to be demonstrated *in vivo* in mouse. Interestingly, RA has recently been shown to be required for differentiation of cephalic NCCs into adipocytes in developing zebrafish embryos (Li et al. 2010), which is a reminiscence of the role of RA in mESC adipogenesis.

It is now well documented that adipose tissues having different impact on the metabolic complications of obesity possess distinct developmental gene signatures suggesting that developmental origins may contribute to adipocyte properties (Tchkonian et al. 2007; Gesta et al. 2006). Therefore, studies on the origins of APs open at least two questions: are adipocytes derived from different developmental origins functionally different? And what are the developmental origins of APs in humans? As lineage tracing is impossible in humans, only specific marker expression could be used to propose an embryonic origin of human samples. *Pax3* expression was shown to be associated with neural crest development in humans and to be maintained in mesenchymal progenies (Betters et al. 2010; Fukuta et al. 2014). *Pax3* thus represents a unique marker to follow the neural crest fate in humans. Interestingly, hiPSC-Brown APs, but not hiPSC-White APs, express *PAX3* and the generation of BAP during hiPSC differentiation is preceded by expression of craniofacial neural crest markers (Hafner unpublished data). *PAX3* expression is maintained in human adults, and microarray analysis revealed that *Pax3* expression marks preferentially UCP1-expressing adipose tissue depots localized in the face (Kouidhi et al. 2015). Altogether, these reports strongly suggest that human BAPs originate from neural crest.

In conclusions, adipogenesis of human pluripotent stem cells is a new area that has tremendous interest to understand how energy-dissipating cells are generated. A thorough understanding of the signals that govern the generation and the differentiation of *PAX3*⁺ cells during pluripotent stem cell development could open new opportunities to better know human brown-like adipocyte ontogenesis and to develop alternative strategies to counteract obesity.

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