Deciphering the Emerging Roles of Adipocytes and Adipose-Derived Stem Cells in Fat Transplantation

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Abstract

Autologous fat transplantation is widely regarded as an increasingly popular method for augmentation or reshaping applications in soft tissue defects. Although the fat transplantation is of simple applicability, low donor site morbidity and excellent biocompatibility, the clinical unpredictability and high resorption rates of the fat grafts remain an inevitable problem. In the sites of fat transplantation, the most essential components are the adipocyte and adipose-derived stem cells (ADSCs). The survival of adipocytes is the direct factor determining fat retention. The efficacy of fat transplantation is reduced by fat absorption and fibrosis due to the inadequate blood flow, adipocyte apoptosis and fat necrosis. ADSCs, a heterogeneous mixture of cells in adipose tissue, are closely related to tissue survival. ADSCs exhibit the ability of multilineage differentiation and remarkable paracrine activity, which is crucial for graft survival. This article will review the recent existing research on the mechanisms of adipocytes and ADSCs in fat transplantation, especially including adipocyte apoptosis, mature adipocyte dedifferentiation, adipocyte browning, ADSCs adipogenic differentiation and ADSCs angiogenesis. The in-depth understanding of the survival mechanism will be extremely valuable for achieving the desired filling effects.

Keywords

adipocyte, fat transplantation, adipose-derived stem cells, apoptosis, adipogenic differentiation, angiogenesis

Introduction

Adipose tissues, composed of mature adipocytes, adipose-derived stem cells (ADSCs), stromal cells, vascular endothelial cells and various immune cells, attract tons of attention in medical research recently[1]. Adipose tissue, classified into white adipose tissue (WAT) and brown adipose tissue (BAT), is relatively abundant in the human body[2]. WAT, predominately white adipocytes, is most commonly found as subcutaneous tissue around the abdomen, breast, thighs, and waist. Multiple studies have indicated that both WAT and BAT synthesize and secrete numerous bioactive molecules that affect multiple endocrine activities. Adipose tissue also plays an important role in mediating energy homeostasis. Besides, the supporting and filling function of adipose tissue cannot be ignored. Adipose tissue is a soft and malleable tissue, present in large quantities in the body, making it an ideal filler for correcting and reshaping contours and volume defects[3]. In the long term, adipose tissue is a relatively safe material for regenerative medicine with multiple biologically active ingredients.

Autologous fat grafting is a widely recognized operation and well-established utilized to correct the soft tissue contour deformities including breast reconstruction[4], facial filling[5], keloid repair[6], hair regeneration[7], and even to treat wrinkles[8]. Autologous fat grafting possesses many advantages, including simple applicability, low donor site morbidity and excellent biocompatibility[9]. Furthermore, as the grafted tissue is endogenic derived from the patient, the side-effects like immune rejection, allergic reactions and tissue toxicity, do not occur. However, variable absorption

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rates of grafted fat are the biggest problem and lead to repeated procedures and unpredictable outcomes[10]. Thus, the inevitable fat absorption may confuse physicians and constrain further application.

The most concerning components in fat transplantation are the adipocytes and ADSCs. The survival of adipocytes is the direct factor determining fat graft retention, therefore the uncertainty of adipocyte survival makes it difficult to predict postoperative effects[11]. A portion of adipocytes after fat transplantation inevitably undergo apoptosis and necrosis due to ischemia, hypoxia, or excessive stress of the survival environment. One of the most significant reasons is the lack of vascular reconstruction in the transplanted adipose tissue. The sufficient blood supply is able to restore nutritional support, transmit stem cells and reduce oxygen deficit for graft tissue. Timely and effective revascularization is beneficial for functional recovery in fat grafting and filling. BAT can utilize fuel such as fatty acids (FAs) to balance body temperature by thermogenesis, with the ability to dissipate energy to produce heat. Interestingly, the induced WAT browning or BAT is believed to promote graft survival. Besides, adipocyte dedifferentiation, accompanied by the downregulation of adipogenic markers, might serve as seed cells to increase the retention rate of fat grafts.

ADSCs exhibit the abilities of multilineage differentiation and remarkable paracrine activity, which are essential for fat transplantation. ADSCs can differentiate into adipocytes, endothelial cells, chondrocytes, bone cells and smooth muscle cells[12]. The efficacy of autologous fat transplantation is reduced by fat absorption and fibrosis due to the inadequate blood flow the fat necrosis. However, ADSCs promotes vascular network formation and stabilization through differentiation[13]. ADSCs are easily accessible from various fat depots and show intrinsic plasticity in giving rise to cell types involved in fat transplantation and angiogenesis. By common consent, the number of ADSCs in adipose tissue is closely related to fat graft survival. Therefore, the maintenance of ADSCs properties during fat transplantation is of great importance and remains highly critical to address.

In plastic surgery, the maneuverability and safety of autologous fat transplantation have become increasingly recognized and continuously improved. Unveiling the substantive characteristics of fat survival remains vital for optimal effect. This article will review the recent existing research on the mechanisms of adipocytes and ADSCs in fat transplantation, especially including adipocyte apoptosis, mature adipocyte dedifferentiation, adipocyte browning, ADSCs adipogenic differentiation and ADSCs angiogenesis (Fig. 1). An in-depth understanding of the mechanisms of adipocytes and ADSCs in fat transplantation will provide better insight into achieving the desired fat filling in graft remodeling.

Adipocyte Apoptosis

Apoptosis, a highly regulated death-dependent process, is critical for mammalian tissue homeostasis, and its disruption has been linked to a wide variety of disorders, including diabetes, obesity, and fat transplantation[14]. The variable absorption rate is one of the most important issues of fat grafting and one of the primary causes of fat graft volume loss is apoptosis. Notably, mechanical damage, ischemia and hypoxia, cell rupture and death, and the release of numerous reactive oxygen species can eventually lead to apoptosis, during adipose tissue extraction and the fat transplantation process[15]. Maintaining apoptosis on a low level in adipocytes, ADSCs and other cells in the fat graft is mandatory to increase graft survival.

Adipocytes represent the major cell type in absorbed cell type in the fat graft. In the model of the fat implant to the dorsal area of rats, Güney et al. verified that the adipocyte apoptosis could be inhibited by utilizing minocycline, thereby increased fat graft survival characterized by bigger graft volume, better fat structures coupled with more vascular[16]. And this apoptosis could be antagonized by synthetic glucocorticoid and insulin-like growth factor-1 (IGF-1)[17]. TNFα-induced apoptosis and gene expression are critical in human adipocyte apoptosis. An in vitro study demonstrated 3T3-L1 adipocyte apoptosis induced by TNF-α was essentially regulated via controlling NF-κB activation, by targeting the TANK gene[18]. Adiponectin is an important adipocyte-secreted adipokine. Liu et al. suggested that adiponectin inhibited endoplasmic reticulum stress-induced adipocyte apoptosis in vivo and in vitro by activating the AMPK/PPARα/ATF2 pathway[19]. It is worth noting that coenzyme Q10 could rescue the adipocytes collected for autologous lipofilling from stress-induced apoptotic death, benefiting for harvesting dynamic adipose tissue[20]. In response to cellular stress, the p38 pathway can lead to apoptosis and can negatively regulate cell proliferation. Nevertheless, contrary to the result of Filson et al., inhibition of p38 significantly increased fat graft apoptosis and absorption[21]. It might be of interest that there is a negative feedback pathway or dose-related response that the inhibitor may act differently on the numerous different cell types and lineages making up the fat graft. The metabolic variations in adipose-specific mevalonate pathway-disrupted (aKO) mice were dramatically reversed after fat transplantation. Thus, apoptosis was essential for adipocytes death induced by HMGCR down-regulation[22]. These studies provide persuasive evidence that the survival rate of fat transplantation can be improved by protecting adipocytes from apoptosis, for better understanding on graft survival theory[23]. The classical graft survival theory considered that certain stubborn adipocytes in the fat graft receive an early and suitable circulation and continue to survive, whereas the remainder of the graft degenerates and is gradually eliminated. As a consequence, inhibition of adipocyte apoptosis can significantly reduce the death of adipocytes and improve the survival rate of adipocytes after fat transplantation. Understanding more about the process of adipocyte apoptosis becomes ever more important in light of newly discovered deregulation of the
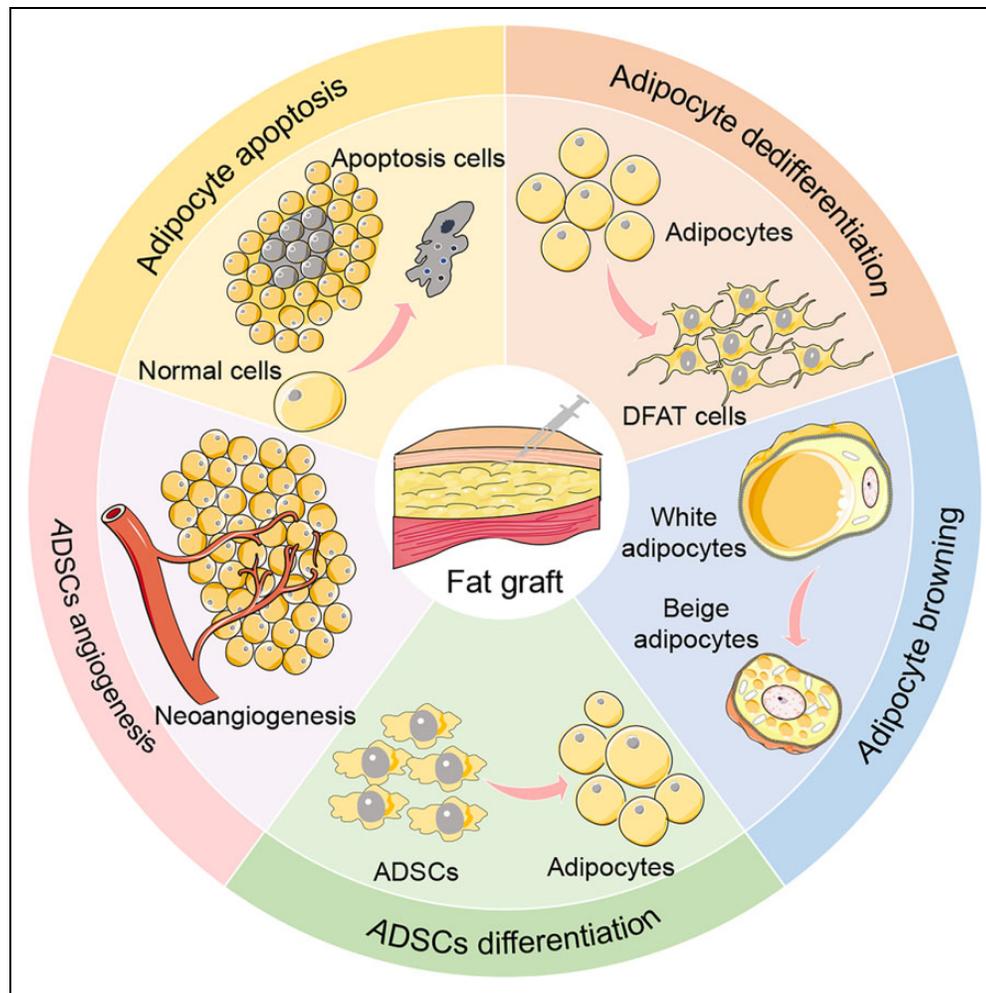
Figure 1. The mechanisms of adipocytes and ADSCs in fat transplantation. Adipocyte apoptosis is the primary causes of fat graft volume loss, resulted in variable absorption rate; Mature adipocyte dedifferentiation increase the retention rate of fat grafts by acting as seed cells; Adipocyte browning may better tolerate avascular environments and improve graft survival; ADSCs adipogenic differentiation is regulated by multiple transcription factors, miRNA and LncRNA; ADSCs have a prominent pro-vascularization effect via vascular endothelial cell differentiation and pro-angiogenic secret ability; ADSCs, adipose-derived stem cells.

apoptotic program contributing and might be served as a therapeutic strategy for shaping fat transplantation.

Abundant ADSCs can be obtained during liposuction. The grafted ADSCs can be replaced by connective tissue or survive and differentiate at the recipient site. ADSCs apoptosis is also an early incident that contributes to transplanted fat tissue depletion and eventual absorption. Li et al. concluded that pre-inhibiting autophagy enhanced the effects of AMPK inhibition on oxygen-glucose deprivation-induced apoptosis and lowered adipogenesis ischemia-challenged ADSCs, presuming that ADSCs apoptosis may differ contextually depending on the specific signaling alteration[24]. Similarly, Shi et al. showed that the spontaneous apoptosis of ADSCs and apoptosis induced by oxidative stress was mediated by MEG3. The result highlighted the role of MEG3 in the apoptosis of ADSCs for further improving the survival rate[25]. The suppression of apoptosis of ADSCs may be attributed to decreased

apoptotic rate and increased cell numbers in tissues, thereby facilitating the survival rate of transplanted adipose tissue. In total, based on the widespread idea that the number of viable adipocytes in a graft correlates with the ultimate volume of persisting fat, an antiapoptotic strategy is essential to remodel the fat behavior and establish favorable retention.

Adipocyte Dedifferentiation

Mature adipocytes were long considered to be terminally differentiated cells that cannot proliferate, and consequently, their role in the regeneration of adipose tissue was ignored. Surprisingly, under appropriate conditions, mature adipocytes, mature adipocytes can reversibly change their phenotype with fibroblast-like morphology and contain multilocular lipid droplets, namely, dedifferentiated fat (DFAT) cells. It was documented that DFAT cells may differentiate into multiple cell lineages, such as skeletal and

smooth muscle cells, cardiomyocytes, osteoblasts, and adipocytes. Additionally, recent studies showed the ability of isolated mature adipocytes to dedifferentiate in vivo and the capacity of the progeny cells to differentiate into mature adipocytes. Adipocytes can be divided into small adipocytes with a diameter of less than 40 μm and large adipocytes with a diameter of 40–100 μm. Tsurumachi et al. proposed that small adipocytes have stronger dedifferentiation ability and could tolerate hypoxia better after fat grafting compared with large adipocytes[26]. Dedifferentiated adipocytes increase the retention rate of fat grafts by acting as seed cells[27].

Many efforts are being made devoted to induced DFAT cells, including mechanical signal induction, cold exposure, hypoxia, and stress induction. Duarte et al. have shown that mechanically separated fat can form DFAT cells in the body and redifferentiate into mature adipocytes, increasing fat content[28]. Dedifferentiated adipocytes are classically obtained by ceiling culture, in which cells are placed in a flask filled with medium to create an air-free environment[29]. In addition, Deng et al. found that under cold stimulation, the expression of adipogenesis-related genes FABP4 and leptin in adipocytes was down-regulated, while the expression of mitochondrial uncoupling-related genes UCP1, PGC-1α, and PRDM16 were up-regulated[30]. The dedifferentiation efficiency of adipocytes toward multipotent DFAT cells could be promoted via periodic exposure to cold in vitro[30]. The results of Banyard showed that the expression of CD34, CD13, CD73, and CD146 related ADSCs markers of DFAT cells in nanofat increased significantly under stress induction[31].

A study collected the gene chips for adipogenic cells and dedifferentiated adipocytes and found that 1590 and 896 kinds of genes were up-regulated and down-regulated, respectively. This result concluded that genes related to focal adhesion and actin cytoskeleton regulation increased significantly during the dedifferentiation of adipocytes[32]. Ma et al. used tissue expanders to pretreat adipose flaps, to increase the retention rate after the fat graft, hereby confirming that mature adipocytes became smaller and multilocular lipid droplets in morphology and underwent dedifferentiation in expanded adipose flaps[27]. These dedifferentiated adipocytes might increase the retention rate of fat grafts functioning as seed cells, together with increased VEGF expression and wider surviving/regenerating zones. Compared with ADSCs and bone mesenchymal stem cells (BMSCs), DFAT cells possess advantages in homogeneity, proliferation ability, adipogenic differentiation ability and lower age requirements for the donor. The relative low dedifferentiation efficiency of mature adipocytes with the established protocols limits their widespread applications. It is necessary to establish an efficient extraction and amplification method for DFAT cells, leading to an increased retention rate of fat grafts.

Adipocyte Browning

Mammals have two main types of adipose tissue: white adipose tissue (WAT), and brown adipose tissue (BAT), each of which possesses unique cell-autonomous properties[33]. WAT mainly accumulates excess energy in the form of triacylglycerol, while BAT directly dissipates energy in the form of heat[34]. Brown fat cells have unique thermogenic properties, which can change the balance between energy intake and energy consumption, thereby affecting the metabolic state[35]. Brown fat is produced after white fat transplantation adapt to tissue remodeling after transplantation, and this change can help repair fat tissue[36]. Following transplantation, adipocytes are exposed to hypoxia and an avascular environment. Oxygen diffusion is essential to the survival of fat graft, concerning about surface-to-volume ratio[37]. The brown adipocytes with a higher surface-to-volume ratio that can facilitate the process of diffusion, resulting in favorable graft survival[38,39].

Manipulation of adipocytes functions with a bona fide angiogenic factor, such as VEGF-A, can significantly improve the survival rate and volume retention of fat grafts and can convey metabolically favorable properties on the recipient[40]. Park's results indicated that the transfer of subcutaneous adipose tissues taken from VEGF-A overexpressing mice into diet-induced obese mice resulted in systemic metabolic benefits, which were associated with improved survival of adipocytes and a concomitant reduced inflammatory response[40]. This opens up new research areas in exploiting fat grafting in metabolic diseases. Hoppela et al. transplanted inactive white fat into muscles, founding that grafting of metabolically inactive fat intramuscularly may induce browning of fat grafts toward more active beige adipose tissue[41]. Cai et al. showed that by using the tamoxifen pre-treated adipocytes in subcutaneous fat transplantation, the adipocytes exhibited the brown fat characteristics with smaller volume, multi-room lipid droplets, and up-regulated UCP-1 expression, which greatly improved fat graft survival[39].

It is recently reported that transplantation of brown adipocytes mixing with matrigel into mice resulted in surprisingly good survival of adipocytes and affected glucose homeostasis[42]. Therefore, pro-angiogenic conditions drive the proliferation of human beige adipocytes progenitors. Thus, white adipocytes browning may better tolerate avascular environments and improve graft survival. Although the current animal researches have highlighted the contribution of beige adipocytes in the repair of adipose tissue after transplantation, it is still necessary to conduct clinical verification to prove whether this is a general trend of fat response to grafting. Additionally, future studies discovering the cellular dynamics of the browning process and its role in the postoperative survival of fat, which will provide further clues to therapeutic targets.

Table 1. Effects of Non-Coding RNAs on Adipogenic Differentiation of ADSCs.

ncRNAs		Mechanism	Model system	Ref.
MiRNAs	miR-150	miR-150 regulated adipogenic differentiation of ADSCs, likely mediated by the downregulation of Notch3	miR-150 knockout mice	49
	miR-378	MiR-378 could promote the subcutaneous lipogenesis	miRNA microarray of human	50
	miR-143	MiR-143 played the modulational role of ADSCs adipogenic differentiation by directly repressing MAP2K5	Rat ADSCs	51
	miR-31, miR-125b-5p and miR-326	The expression of miR-31, miR-125b-5p, and miR-326 were downregulation in the adipogenic differentiation process	Rat ADSCs	52
	miR-450a-5p	MiR-450a-5p promoted lipogenesis by inhibiting the expression of WISP2	Rat ADSCs	53
LncRNAs	lncRNA-Adi	LncRNA-Adi was highly expressed in ADSCs and promoted adipogenic differentiation of ADSCs	Rat ADSCs	54
	LncRNA TINCR	LncRNA TINCR promoted adipose differentiation of hADSCs by inhibiting miR-31	Human ADSCs	55
	LncRNA H19	LncRNA H19 knockdown suppressed while miR-30a inhibition promoted the mRNA expression and the protein levels of C8orf4 and adipogenic differentiation	Human ADSCs	56

ncRNAs, non-coding RNAs; ADSCs, adipose-derived stem cells; miRNA, microRNA; LncRNA, long non-coding RNA; MAP2K5, mitogen-activated protein kinase 5, also named MEK5; WISP2, WNT1 inducible signaling pathway protein 2.

ADSCs Adipogenic Differentiation

The fat formation is essential for the long-term retention of fat graft. The classical "three-zone" theory emphasizes the significance that the optimal survival of fat graft depends largely on adipose tissue regeneration after fat grafting[43]. It is noteworthy that mesenchymal stem cells (MSCs), such as ADSCs and BMSCs, are probably the seed cells for adipogenesis, attributing to their directional differentiation to a wide variety of cell lineages by adjusting cultivating methods[44]. As a critical MSC-specific property, differentiation potential fate is synergistically influenced by the transcriptome, proteome, immunophenotype, and immunomodulatory activities[45]. MSCs, possessing a fibroblastic morphology, is a heterogeneous population derived from multiple tissues, including peripheral blood, synovium, bone marrow, and adipose tissue. Most remarkably, ADSCs are of multilineage differentiation with abundant source, high yield, easy obtainment, and paracrine function, raising practical advantages in clinical medicine, particularly in fat transplantation[46]. Cell-assisted lipotransfer (CAL) is a process in which fat grafting is supplemented with autologous ADSCs, benefiting for the improved survival rate of the fat graft with less fat resorption and a marked decrease in the adverse effects of lipo injection[47]. The mechanism of CAL is concerning lipogenic differentiation, angiogenesis ability and endocrine function of ADSCs. As the excellent adipogenic differentiation potential, ADSCs and BMSCs have attracted increasing attention in fat transplantation.

Epigenetics, represented by miRNAs and LncRNAs, are an important factor in regulating ADSC adipogenic differentiation (Table 1). miRNAs are members of the non-coding small RNA family, which regulates gene expression by inhibiting mRNA translation or promoting miRNA degradation at the post-transcriptional level[48]. Notably, an increasing number of miRNAs have been revealed to play a pivotal role in the maintenance of stemness and modulation of mobilization, proliferation, and differentiation of ADSCs, either promoting or inhibiting adipogenesis. Li et al. investigated the mechanism of miR-150 in adipogenic differentiation of ADSCs[49]. The experiment results indicated that miR-150 was expressed in ADSCs and was critical for adipogenesis by targeting Notch3. Yu et al. Performed micro-array analysis of miRNAs regulated by peroxisome proliferator-activated receptor gamma (PPARγ) in human adipocytes, and found that the expression of 27 miRNAs changed. Among them, miR-378 could promote the adipogenesis of subcutaneous adipocytes but not visceral adipocytes[50]. Chen et al. pointed out that miR-143 exerted its adipogenic effect by regulating MAP2K5. The increased expression of miR-143 during cell expansion could inhibit adipogenic differentiation of ADSCs, while overexpression during growth arrest or terminal differentiation promotes differentiation[51]. In addition, the expression of miR-31, miR-125b-5p, and miR-326 was deregulated in rat ADSCs during adipogenic differentiation, indicating their potential role in adipogenic differentiation[52]. Patel showed that adipose tissue-derived exosomes contain 45 kinds of miRNAs, of which miR-450a-5p was one of the most abundant miRNAs, which promoted lipogenesis by inhibiting the expression of WISP2[53]. Regulating specific miRNAs can promote ADSCs lipogenesis, which may be a novel and effective method to increase the retention rate of fat transplantation.

The concept that LncRNAs play an indispensable function in adipogenesis processes has been gradually verified. Chen et al. found the high expression of lncRNA-Adi in adipogenic-induced ADSCs[54]. LncRNA-Adi could

competitively interact with miR-449a to influence CDK6-pRb-E2F1 axis activation, which was crucial in the regulation of cell division and PPARγ expression during adipogenesis[54]. Another research also revealed that the LncRNA TINCR served as a ceRNA for miR-31 in hADSCs to downregulate miR-31 and upregulate miR-31 downstream target C/EBP-α and thereby formed a feedback loop, ultimately leading to modulate adipogenic differentiation in human ADSCs[55]. LncRNA H19 was related to multiple cellular differentiation, including modulating adipogenic differentiation, the mechanism of which was the role of H19 serving as a ceRNA to compete with C8orf4 for miR-30a binding[56]. From the perspective of LncRNA-miRNA-mRNA regulation, it provides a novel regulatory mechanism of ADSCs adipogenic differentiation.

Autophagy, a highly conserved homeostatic mechanism necessary for cell survival, is involving autolysosomal degradation of cellular components, such as protein aggregates, damaged organelles and even various pathogens[57]. Autophagy plays a vital role in ADSCs adipogenic differentiation and is implicated in the fat transplantation. Obese people have a lot of fat, and liposuction parts are usually fat-rich parts[58]. Therefore, the fat environment is an influencing factor for fat transplantation. For example, the hypertrophic adipocytes of obese patients, exposed to a mildly inflammatory environment, is adjusting the cellular function in response to numerous cellular stressors. Evidence suggests that the level of adipocyte autophagy was increased in obese and high-fat environments. In an in vitro model used 3T3-L1 adipocytes, Yin et al. found that the JNK-dependent activation of autophagy diminished palmitate-induced inflammation by inhibiting the expression of monocyte chemoattractant protein-1 (MCP-1) and IL-6[59].

The autophagy level of donor adipocytes is influenced to a certain extent under the circumstance of fat transplantation with ischemia, hypoxia, and inflammatory features. Although at present, few studies have reported the autophagy role in fat transplantation, we speculate that autophagy can impact on the fat transplantation by intervene the behavior of ADSCs and adipocytes. Ischemia is one of the main causes of the high rate of absorption of transplanted autologous fat[60]. The apoptosis and autophagy of ADSCs are involved in the process of programmed cell death induced by the ischemic and mitogen-deprived microenvironments[61]. Li et al. treated the ADSCs exposed to oxygen-glucose deprivation (OGD), which was a simulated ischemic microenvironment[62]. During adipogenesis, autophagy inhibition decreased the expression of oil droplet accumulation, lipoprotein lipase, and PPARγ protein in ADSCs with or without the OGD challenge[62]. Upregulating autophagy may promote the survival and adipogenesis of ischemia-challenged ADSCs. As autophagy has well-established properties in ADSCs adipogenic differentiation, understanding the complex relationships of autophagy and how ADSCs and adipocytes are affected by autophagy would attenuate adipocytes

dysfunction and inflammation, potentially benefiting for the efficacy of fat transplantation.

Besides, the adipogenic differentiation process of ADSCs is governed by various transcription factors (TFs), such as EBF family (EBF1, EBF2, EBF3), PPARs, C/EBPα, CREB, KLF5, KLF15, STAT5a, and ATF4[63,64]. Huang et al. investigated underlying mechanisms on adipogenic differentiation using rat ADSCs and the mouse pre-adipocyte cell line 3T3-L1, showing that adipogenic differentiation was promoted with PEDF knockdown by modulating downstream effector CD36 through ATGL, in a PPARγ-dependent manner[65]. More interestingly, the results of an animal study of fat transplantation with ADSCs, insulin could enhance the survival and differentiation of ADSCs within nonvascularized fat grafts[66]. Wang et al. indicated that exogenous IGF2 supplementation enhanced proliferation and multilineage differentiation of ADSCs accompanied by the expression of the stemness-related markers NANOG, OCT4, and SOX2. This proposed a new insight into the method for culturing ADSCs and may facilitate the ADSCs based tissue engineering and therapy[67]. At present, three theories (Fig. 2) have been proposed to describe how fat grafts survive avascular graft surgery, including graft survival theory, graft replacement theory and host cell replacement theory[60]. Meanwhile, both graft replacement theory and host replacement theory claim that very few donor adipocytes survive the grafting process. Graft replacement theory stated that grafted adipocytes were largely replaced by the donor ADSCs that were concurrently transferred in the graft. Instead, according to host cell replacement theory, no grafted cells survived, and all the cells were replaced by recipient cells[68]. Conformably, the mechanisms of fat graft survival are closely related to ADSCs, which highlighted the importance of ADSCs in the grafted fat for adipocytes regeneration. Thus mechanistically, miRNA, LncRNA, TFs, and autophagy are engaged in influencing the adipogenic differentiation capabilities of ADSCs, representing attractive targets for satisfactory fat morphology.

BMSCs, defined as a class of adult stem cells with self-renewal and multidirectional differentiation potential, plays an important role in regulating the homeostasis and maintenance of stem cells in the microenvironment. The adipogenic and osteogenic function of BMSCs have always been the focus of research in the field of cell transplantation and tissue engineering. Current data supported that miR-199a-3p improved the adipogenic differentiation of BMSCs by inhibiting KDM6A and sequential downstream inactivation of the WNT signaling pathway, uncovering a novel mechanism underlying adipogenic differentiation and highlighting a potential target for osteoporotic disease therapy[69]. In a CAL rabbit model, Xing et al. proposed an interesting method using bone marrow aspirate and bone marrow concentrate including BMSCs, confirmed the implanted BMSCs contribute to fat graft survival by multilineage differentiation, adipogenesis and angiogenesis[70]. BMSC-assisted fat graft is effective and safe for soft tissue augmentation and may be
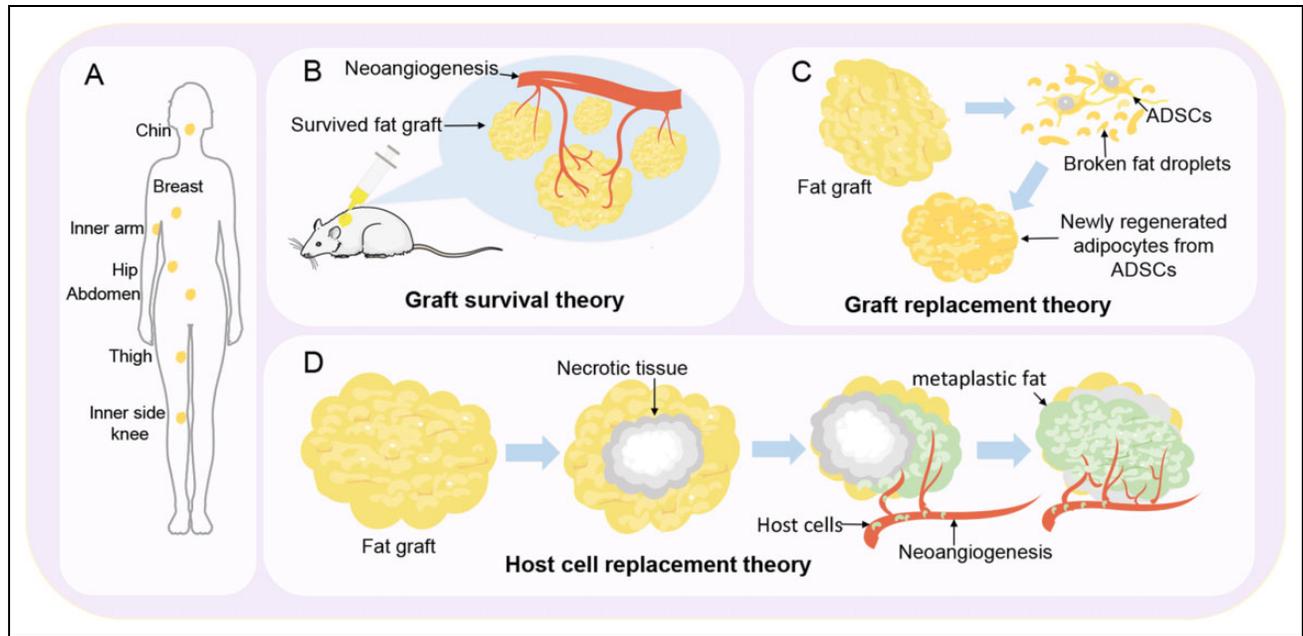
Figure 2. Theories of fat graft survival. (A) The body fat distribution differs from respective zones coupled with different cell viability. (B) Graft survival theory: certain stubborn adipocytes in the fat graft receive an early and suitable circulation and continue to survive, whereas the remainder of the graft degenerates and is gradually eliminated. (C) Graft replacement theory: most of the adipocytes die after transplantation and adipocytes in the regenerative area do survive through graft-derived ADSCs adipogenic differentiation. (D) Host cell replacement theory: transplanted fat is completely necrotic and is replaced by fibrous tissue or newly formed metaplastic fat. Adipose-derived stem cells, ADSCs.

superior to conventional lipo-injection[71]. Previous studies have also demonstrated that BMSC-derived extracellular vesicles (BMSC-EVs) may promote neovascularization by stimulating the secretion of proangiogenic factors for improving the long–term retention and quality of transplanted fat[72].

Existing studies have shown that the adipogenic differentiation function of MSCs can effectively improve the survival rate of transplants, but ADSCs are applied much more frequently than BMSCs. However, in lean or elderly people and patients with autoimmune-related diseases, the biological activity of ADSCs is affected by these factors and the quantity of ADSCs is also sharply declined due to the reduced fat tissue, which limits the utilization of ADSCs in fat transplantation. Therefore, under some circumstances, BMSCs are potential alternatives in CAL, complemented by ADSCs.

ADSCs Angiogenesis

Adipose tissue has a special tissue structure and blood supply system. In the mature adipose tissue, only a few monocellular fat cells, like grape clusters, are arranged on the vascular scaffold, but they occupy most of the space and volume, which requires high energy consumption and high blood supply. In the early stage of fat transplantation, the adipose tissue mainly relies on infiltration and osmosis to maintain the nutrition supply[73]. The presence of more fat

droplets than capillary recipient sites will lead to insufficient neoangiogenesis. However, emerging evidence has shown that the efficacy of fat transplantation is reduced by fat absorption and fibrosis that are closely related to insufficient vascularization. ADSCs are considered to be a class of MSCs derived from vascular matrix components in adipose tissue, with self-renewal, multiple differentiation potentials, and powerful paracrine functions. In fat transplantation, ADSCs have a prominent pro-vascularization effect via vascular endothelial cell differentiation and pro-angiogenic secret ability.

ADSCs can directly or indirectly differentiate into endothelial cells, smooth muscle cells, and pericytes to promote the formation and stabilization of vascular networks and improve the survival rate of fat transplantation. Increasing evidence suggests that ADSCs with other types of MSCs, are anatomically and functionally associated with vascular and perivascular habitats in various tissues. Harris et al. confirmed that the co-implantation of fat lipoaspirate with ADSCs differentiated toward an endothelial phenotype observably improved both survival and neovascularization of the transplanted fat lipoaspirate[74]. An interesting study demonstrated that CD146 positive cells selected from a heterogeneous mixture of ADSCs expressed higher levels of the adipocyte markers adiponectin and leptin, and possessed more favorable angiogenic and adipogenic properties, which might provide significant benefits for reconstructive and tissue-engineering applications in fat transplantation[75].

Hong et al. showed that newly differentiated fat from donor ADSCs and recipient tissue integrated with survived donor fat resulted in a quantitative difference in angiogenesis and adipogenesis during adipose remodeling according to the concentration of ADSCs[76].

Interestingly, Yuan et al. proposed that early vascularization of ADSCs mainly relies on secretion to produce various vascularization factors, rather than differentiation to produce functional cells[77]. As also confirmed by Yi et al., the role of exogenous ADSCs in free fat transplantation may not directly participate in angiogenesis and adipogenesis but may promote the survival ratio of the graft-resident interstitial cells, which are involved in angiogenesis and adipogenesis, via a paracrine effect[78]. Importantly, ADSCs also participate in enhancing neovascularization via the autocrine and paracrine effect of angiogenic cytokines, including VEGF, bFGF, EGF, IGF-1, HGF, PDGF, and thereby increase the survival rate of fat grafts. VEGF, which stimulates endothelial cell migration and proliferation, regulates graft angiogenesis during fat transplantation. it was consistent with the research of Chen et al. that the VEGF production significantly stimulated by CRP, mainly by activating HIF-1α via the CD64/PI3K/Akt and MAPK/ERK signal pathways in ADSCs, implicating that CRP might have a significant effect on vasa vasorum growth by activating the proangiogenic function of ADSCs[79]. Besides, another possible mechanism of promoting the ADSCs angiogenesis, was that HIF1A suppressed the expression of miR-20a via targeting its promoter region[80]

Growing evidence has revealed that ADSCs possess the ability to enhance vascularization, partly due to extracellular vesicles (EVs). In a long-term nude mouse fat transplantation model, Mou et al. demonstrated that ADSCs-derived EVs could enhance the volume retention of fat grafts by improving angiogenesis and regulating immune responses[81]. Furthermore, the study found that ADSC-Exos can effectively promote the survival of graft, neovascularization and attenuated inflammation in the fat grafts. Hypoxia treatment could further enhance the beneficial effect of ADSC-Exos[82]. Hoseini et al. transfected plasmids encoding FGF-1 into ADSCs for transplantation and culture, demonstrating that FGF-1 can promote vascular proliferation and tube formation in fat transplantation[83]. Thus, as the unsatisfactory blood supply is a very important obstacle for fat graft survival, utilize the properties of vascular angiogenic differentiation and proangiogenic secret ability, which will be a benefit for protecting fat grafted from the inadequate blood supply. These findings, based on ADSCs-derived extracellular vesicles, could offer a promising addition or alternative to autologous fat grafting once the clinical translation to the patient shows sufficient effectiveness, and may potentially show its regenerative potential for future clinical applications.

Conclusions and Perspectives

Adipose tissue transplantation is regarded as a safe and advantageous procedure for augmentation or reshaping applications in different clinical situations. In summary, the adipocytes and ADSCs are extremely involved in the survival process of fat graft, including adipocyte apoptosis, dedifferentiation of mature adipocytes, white adipocyte browning, ADSCs adipogenic differentiation and ADSCs angiogenesis. These lately theoretical studies will support to solve the problem of fat regeneration and absorption. As these mechanisms are explored, fat transfer techniques are being innovated. The understanding of the ischemia and hypoxia microenvironment of adipocytes after transplantation will be beneficial to improve the details of approaches of fat transplantation, involving in liposuction methods, fat pre-treatments, injection site and frequency, for the purpose of sustaining high adipocytes viability. The properties of ADSCs with self-renewal and multilineage differentiation, make ADSCs to be attractive candidates fat transplantation and ADSCs-based engineering biomaterials[84].

Nowadays, as the fat absorption is an inevitable event, the filling effect of fat transplantation is still not fairly satisfactory. The reduction in tissue volume often leads to depressions, asymmetric morphology and even failure of the surgical filling. The reasons may attribute to the following issues: (i) The clinical unpredictability of autologous fat grafting originates partially from the unique characteristics of adipose tissue. The adipose tissues from different parts of the body are not equivalent. Besides, the character of the same site of fat also differs from a different individual[85]. To a certain extent, these factors determine the ending of adipocytes and the effect of graft remodeling. (ii) The network of the graft is rather complex, which influence on the adipocytes survival. Firstly, a variety of immune cells such as neutrophils and macrophages are capable of promoting fat graft survival, such as removing dead cells, grease, promoting angiogenesis, regulating adipocytes metabolism and so on[86,87]. Secondly, the graft cells, like ADSCs, can secrete various cytokines involved in the regulation of immune cell function and adipocytes viability. These multiple cytokines can stimulate diverse biological effects synergistically rather than independently in fat transplantation[88]. Thus, immune regulation is not the sole function of any cell, and all these cells collectively establish a complex regulatory network through mutual promotion or restriction, resulting in significant individual differences in the effect of fat transplantation. (iii) The clinical fat transplantation is not strictly standardized. The fat retention rate also depends on the technology, experience of physicians and equipments. Several standard protocols are available for fat grafting based on simple procedures such as lipoaspirate, gravity separation, and filtration or centrifugation to remove impurities and obtain relatively pure adipocytes. However, the lack of standardization in harvesting and injection protocols, combined with the unpredictability of the resorption rate, pose a

significant limitation for graft retention. New and standardized approaches are needed to make fat cells not only accessible functional, but also more efficient and functional.

There are still some problems remained to be an urgent clinical challenge of fat transplantation. It is an interesting issue that generally, the grafted adipocytes injected in facial sites manifest higher survival that in the breast. This fact proposes a research point that the fat transplantation with smaller fragments is better than that with larger fat volume. The methods to sustain the optimal morphologic change in the face and ameliorate the graft survival in breast, will be of considerable value. Another important issue is the safety of fat transplantation. Although autologous fat grafting is becoming a widely popularized technique for breast reconstruction in breast cancer patients who have undergone a mastectomy, the emerging evidence of cancer-associated adipocytes involved in oncological safety still deserves consideration. Due to fat necrosis and calcification in breast tissue, fat transplantation may complicate breast imaging and breast cancer surveillance. It is necessary to conduct clinical trials on a large scale and with long-term follow-up in order to ensure the oncologic safety of fat grafting after mastectomy. More significantly, after autologous fat transplantation, the fat tissue undergoes necrotic and is reabsorbed over time, with the unpredictable survival rate of autologous adipocytes of the intraoperative filling volume[89]. There will still be an urgent requirement of avoiding adipocytes absorption. By the combination of multiple assisted technology, such as platelet rich plasma (PRP), platelet rich fibrin (PRF), nanofat, CAL, exposure of physical factors or cytokine stimulation, or nano-materials, it will contribute to the optimal morphological adjustment.

In conclusion, adipose tissue is a relatively safe tissue of filling substance in conformance with that fat transplantation is a relatively safe, efficient, common procedure in plastic surgery, but still with a major limitation in its unpredictable graft retention. There will still be a matter of implementing mechanisms and technology studies in depth for achieving the desired filling effects of fat.

Authors Note

Yi Yi, and Weijie Hu authors contributed equally to this work.

Author Contributions

YY and WH performed literature search and wrote the manuscript. QZ and YW conceived the project and revised the manuscript. CZ, MW, HZ, MX, and WL edited the manuscript. All authors reviewed the manuscript and all approved of the final version.

Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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References

1. Eckel-Mahan K, Ribas Latre A, Kolonin MG. Adipose stromal cell expansion and exhaustion: mechanisms and consequences. Cells. 2020;9(4):863.
2. Divakaran SJ, Srivastava S, Jahagirdar A, Rajendran R, Sukhdeo SV, Rajakumari S. Sesaminol induces brown and beige adipocyte formation through suppression of myogenic program. FASEB J. 2020;34(5):6854–6870.
3. Xue EY, Narvaez L, Chu CK, Hanson SE. Fat processing techniques. Semin Plast Surg. 2020;34(01):011–016.
4. Davis MJ, Perdanasari AT, Abu-Ghname A, Gonzalez SR, Chamata E, Rammos CK, Winocour SJ. Application of fat grafting in cosmetic breast surgery. Semin Plast Surg. 2020; 34(01):024–029.
5. Lin Z, Yang K, Li G, Wei S, Liu Y. Efficacy and safety of subcutaneous temporal autologous micro-fat augmentation. Aesthetic Plast Surg. 2020;44(6):2098–2106.
6. Onur Erol O, Agaoglu G, Jawad MA. Combined non-ablative laser and microfat grafting for burn scar treatment. Aesthetic Surg J. 2019;39(4):NP55–NP67.
7. Kuka G, Epstein J, Aronowitz J, Glasgold MJ, Rogal JG, Brown W, Geronemus RG, Daniels EJ, Washenik K. Cell enriched autologous fat grafts to follicular niche improves hair regrowth in early androgenetic alopecia. Aesthetic Surg J. 2020;40(6):NP328–NP339.
8. Kim K, Fan Y, Lin G, Park YK, Pak CS, Jeong JH, Kim S. Synergistic effect of adipose-derived stem cells and fat graft on wrinkles in aged mice. Plast Reconstr Surg. 2019;143(6): 1637–1646.
9. Ramot Y, Silyuk T, Murad S, Zlotogorski A. Hirsutism induced by facial autologous fat grafting. Ski Appendage Disord. 2020;6(1):41–43.
10. Lv Q, Li X, Qi Y, Gu Y, Liu Z, Ma G. Volume retention after facial fat grafting and relevant factors: a systematic review and meta-analysis. Aesthetic Plast Surg. 2020.
11. Xiong S, Yi C, Pu LLQ. An overview of principles and new techniques for facial fat grafting. Clin Plast Surg. 2020;47(1): 7–17.
12. Liu G, Pan Y, Liu Y, Hu J, Zhang X, Zhang D, Wang Y, Feng Y, Yu J, Cheng Y. Ghrelin promotes neural differentiation of adipose tissue-derived mesenchymal stem cell via AKT/mTOR and β-catenin signaling pathways. Kaohsiung J Med Sci. 2020; 36(6):405–416.
13. Gontijo-de-Amorim NF, Charles-de-Sá L, Rigotti G. Fat grafting for facial contouring using mechanically stromal vascular fraction–enriched lipotransfer. Clin Plast Surg. 2020;47(1): 99–109.

14. Camargo A, Rangel-Zúñiga OA, Alcalá-Díaz J, Gomez-Delgado F, Delgado-Lista J, García-Carpintero S, Marín C, Almadén Y, Yubero-Serrano EM, López-Moreno J, Tinahones FJ, et al. Dietary fat may modulate adipose tissue homeostasis through the processes of autophagy and apoptosis. Eur J Nutr. 2017;56(4):1621–1628.

15. Abu-Ghname A, Perdanasari AT, Reece EM. Principles and applications of fat grafting in plastic surgery. Semin Plast Surg. 2019;33(03):147–154.

16. Güney K, Tatar S, Özel B, Seymen C, Elmas Ç, Tuncer S, Çenetoğlu S. The effect of minocycline on fat graft survival and apoptotic pathway. Facial Plast Surg. 2019;35(01): 096–102.

17. Niesler C, Urso B, Prins J, Siddle K. IGF-I inhibits apoptosis induced by serum withdrawal, but potentiates TNF-alpha-induced apoptosis, in 3T3-L1 preadipocytes. J Endocrinol. 2000;167(1):165–174.

18. Qi R, Huang J, Wang Q, Liu H, Wang R, Wang J, Yang F. MicroRNA-224-5p regulates adipocyte apoptosis induced by TNFα via controlling NF-κB activation. J Cell Physiol. 2018; 233(2):1236–1246.

19. Liu Z, Gan L, Wu T, Feng F, Luo D, Gu H, Liu S, Sun C. Adiponectin reduces ER stress-induced apoptosis through PPAR α transcriptional regulation of ATF2 in mouse adipose. Cell Death Dis. 2016;7(11):e2487–e2511.

20. Witort EJ, Pattarino J, Papucci L, Schiavone N, Donnini M, Lapucci A, Lulli M, Lo Russo G, Mori A, Dini M, Capaccioli S.Autologous lipofilling: coenzyme q10 can rescue adipocytes from stress-induced apoptotic death. Plast Reconstr Surg. 2007;119(4):1191–1199.

21. Filson SA, Keren A, Goldstein N, Ullmann Y. The Opposite expected effect of p38 inhibitors on fat graft survival. Plast Reconstr Surg Glob Open. 2016;4(7):e806.

22. Yeh Y-S, Jheng H-F, Iwase M, Kim M, Mohri S, Kwon J, Kawarasaki S, Li Y, Takahashi H, Ara T, Nomura W, et al. The Mevalonate pathway is indispensable for adipocyte survival. iScience. 2018;9:175–191.

23. Zhao J, Yi C, Li L, Zheng Y, Wu K, Liang L, Xia W, Guo S. Observations on the survival and neovascularization of fat grafts interchanged between C57BL/6-gfp and C57BL/6 mice. Plast Reconstr Surg. 2012;130(3):398e–406e.

24. Li C, Chen K, Jia M, Ding X, Jiang Z, Li L, Zhang D. AMPK promotes survival and adipogenesis of ischemia-challenged ADSCs in an autophagy-dependent manner. Biochim Biophys Acta Mol Cell Biol Lipids. 2018;1863(12):1498–1510.

25. Shi Y. MEG3 regulates apoptosis of adipose–derived stem cells. Mol Med Rep. 2020;21(6):2435–2442.

26. Tsurumachi N, Akita D, Kano K, Matsumoto T, Toriumi T, Kazama T, Oki Y, Tamura Y, Tonogi M, Isokawa K, Shimizu N, et al. Small buccal fat pad cells have high osteogenic differentiation potential. Tissue Eng Part C Methods. 2016;22(3): 250–259.

27. Ma J, Xia J, Gao J, Lu F, Liao Y. Mechanical signals induce dedifferentiation of mature adipocytes and increase the retention rate of fat grafts. Plast Reconstr Surg. 2019;144(6): 1323–1333.

28. Duarte MS, Bueno R, Silva W, Campos CF, Gionbelli MP, Guimarães SEF, Silva FF, Lopes PS, Hausman GJ, Dodson MV. TRIENNIAL growth and development symposium: dedifferentiated fat cells: potential and perspectives for their use in clinical and animal science purpose. J Anim Sci. 2017;95(5): 2255–2260.

29. Matsumine H, Takeuchi Y, Sasaki R, Kazama T, Kano K, Matsumoto T, Sakurai H, Miyata M, Yamato M. Adipocyte-derived and dedifferentiated fat cells promoting facial nerve regeneration in a rat model. Plast Reconstr Surg. 2014;134(4): 686–697.

30. Deng Z, Zou J, Wang W, Nie Y, Tung W-T, Ma N, Lendlein A. Dedifferentiation of mature adipocytes with periodic exposure to cold. Clin Hemorheol Microcirc. 2019;71(4):415–424.

31. Chaput B, Laloze J, Grolleau J-L, Espagnolle N, Bertheuil N, Varin A. Phenotypic analysis of stromal vascular fraction after mechanical shear reveals stress-induced progenitor populations. Plast Reconstr Surg. 2017;139(4):1024e–1025e.

32. Hu C, Cao H, Pan X, Li J, He J, Pan Q, Xin J, Yu X, Li J, Wang Y, Zhu D, et al. Adipogenic placenta-derived mesenchymal stem cells are not lineage restricted by withdrawing extrinsic factors: developing a novel visual angle in stem cell biology. Cell Death Dis. 2016;7(3):e2141–e2141.

33. Cuevas-Ramos D, Mehta R, Aguilar-Salinas CA. Fibroblast growth factor 21 and browning of white adipose tissue. Front Physiol. 2019;10:37.

34. Lee JH, Park A, Oh KJ, Lee SC, Kim WK, Bae KH. The role of adipose tissue mitochondria: regulation of mitochondrial function for the treatment of metabolic diseases. Int J Mol Sci. 2019;20(19):4924.

35. Pan R, Zhu X, Maretich P, Chen Y. Combating obesity with thermogenic fat: current challenges and advancements. Front Endocrinol (Lausanne). 2020;11:185.

36. Qiu L, Zhang Z, Zheng H, Xiong S, Su Y, Ma X, Yi C. Browning of human subcutaneous adipose tissue after its transplantation in nude mice. Plast Reconstr Surg. 2018;142(2):392–400.

37. Khouri RK, Khouri R-ER, Lujan-Hernandez JR, Khouri KR, Lancerotto L, Orgill DP. Diffusion and Perfusion. Plast Reconstr Surg Glob Open. 2014;2(9):e220.

38. Koseki K, Suzuki H.Deformation dynamics of giant unilamellar vesicles in the large surface-to-volume ratio regime: the emergence of neuron-like morphology. Langmuir. 2020; 36(22):6238–6244.

39. Cai J, Li B, Wang J, Liu K, Zhang Y, Liao Y, Lu F. Tamoxifen-Prefabricated Beige Adipose Tissue Improves Fat Graft Survival in Mice. Plast Reconstr Surg. 2018;141(4):930–940.

40. Park J, Kim M, Sun K, An YA, Gu X, Scherer PE. VEGF-A–expressing adipose tissue shows rapid beiging and enhanced survival after transplantation and confers il-4–independent metabolic improvements. Diabetes. 2017;66(6):1479–1490.

41. Hoppela E, Grönroos TJ, Saarikko AM, Tervala TV., Kauhanen S, Nuutila P, Kivinen K, Hartiala P. Fat grafting can induce browning of white adipose tissue. Plast Reconstr Surg Glob Open. 2018;6(6):e1804.

42. Min SY, Kady J, Nam M, Rojas-Rodriguez R, Berkenwald A, Kim JH, Noh H-L, Kim JK, Cooper MP, Fitzgibbons T, Brehm

MA, et al. Human "brite/beige" adipocytes develop from capillary networks, and their implantation improves metabolic homeostasis in mice. Nat Med. 2016;22(3):312–318.

43. Eto H, Kato H, Suga H, Aoi N, Doi K, Kuno S, Yoshimura K. The Fate of adipocytes after nonvascularized fat grafting. Plast Reconstr Surg. 2012;129(5):1081–1092.

44. Yang H, Liu Y, Liu X, Gu H, Zhang J, Sun C.Concise review: the regulatory mechanism of lysine acetylation in mesenchymal stem cell differentiation. Stem Cells Int. 2020;2020: 7618506.

45. Han Y, Li X, Zhang Y, Han Y, Chang F, Ding J. Mesenchymal stem cells for regenerative medicine. Cells. 2019;8(8):886.

46. Moustaki M, Papadopoulos O, Verikokos C, Karypidis D, Masud D, Kostakis A, Papastefanaki F, Roubelakis MG, Perrea D.Application of adipose-derived stromal cells in fat grafting: basic science and literature review. Exp Ther Med. 2017;14(3): 2415–2423.

47. Bae YC, Kim KH, Yun HJ, Oh CH, Chang JH, Yi CR, Lee JW, Bae SH. A study on the effective ratio of fat to stromal vascular fraction for cell-assisted lipotransfer. Aesthetic Plast Surg. 2020;44(1):162–167.

48. Nepal C, Coolen M, Hadzhiev Y, Cussigh D, Mydel P, Steen VM, Carninci P, Andersen JB, Bally-Cuif L, Müller F, Lenhard B. Transcriptional, post-transcriptional and chromatin-associated regulation of pri-miRNAs, pre-miRNAs and moR-NAs. Nucleic Acids Res. 2016;44(7):3070–3081.

49. Li X, Zhao Y, Li X, Wang Q, Ao Q, Wang X, Tian X, Tong H, Kong D, Chang S, Bai S, et al. MicroRNA-150 modulates adipogenic differentiation of adipose-derived stem cells by targeting notch3. Stem Cells Int. 2019;2019:2743047.

50. Yu J, Kong X, Liu J, Lv Y, Sheng Y, Lv S, Di W, Wang C, Zhang F, Ding G. Expression profiling of PPARγ-regulated microRNAs in human subcutaneous and visceral adipogenesis in both genders. Endocrinology. 2014;155(6):2155–2165.

51. Chen L, Hou J, Ye L, Chen Y, Cui J, Tian W, Li C, Liu L. MicroRNA-143 regulates adipogenesis by modulating the MAP2K5–ERK5 Signaling. Sci Rep. 2015;4(1):3819.

52. Tang Y-F, Zhang Y, Li X-Y, Li C, Tian W, Liu L. Expression of miR-31, miR-125b-5p, and miR-326 in the adipogenic differentiation process of adipose-derived stem cells. OMICS. 2009;13(4):331–336.

53. Zhang Y, Yu M, Dai M, Chen C, Tang Q, Jing W, Wang H, Tian W. miR-450a-5p within rat adipose tissue exosome-like vesicles promotes adipogenic differentiation by targeting WISP2. J Cell Sci. 2017;130(6):1158–1168.

54. Chen Y, Li K, Zhang X, Chen J, Li M, Liu L. The novel long noncoding RNA lncRNA-Adi regulates adipogenesis. Stem Cells Transl Med. 2020;9(9):1053–1067.

55. Liu Y, Wang Y, He X, Zhang S, Wang K, Wu H, Chen L. LncRNA TINCR/miR-31-5p/C/EBP-α feedback loop modulates the adipogenic differentiation process in human adipose tissue-derived mesenchymal stem cells. Stem Cell Res. 2018; 32:35–42.

56. Li K, Wu Y, Yang H, Hong P, Fang X, Hu Y. H19/miR-30a/C8orf4 axis modulates the adipogenic differentiation process

in human adipose tissue-derived mesenchymal stem cells. J Cell Physiol. 2019;234(11):20925–20934.

57. Pompili E, Fabrizi C, Fumagalli L, Fornai F. Autophagy in trimethyltin-induced neurodegeneration. J Neural Transm. 2020;127(7):987–998.

58. Collins PS, Moyer KE. Evidence-based practice in liposuction. Ann Plast Surg. 2018;80(Suppl 6):S403–S405.

59. YIN J, WANG Y, GU L, FAN N, MA Y, PENG Y.Palmitate induces endoplasmic reticulum stress and autophagy in mature adipocytes: implications for apoptosis and inflammation. Int J Mol Med. 2015;35(4):932–940.

60. Pu LLQ. Mechanisms of fat graft survival. Ann Plast Surg. 2016;77(1):S84–S86.

61. Tao J, Wang H, Zhai Y, Park H, Wang J, Ji F, Zhang Z. Down-regulation of Nrf2 promotes autophagy-dependent osteoblastic differentiation of adipose-derived mesenchymal stem cells. Exp Cell Res. 2016;349(2):221–229.

62. Li C, Ye L, Yang L, Yu X, He Y, Chen Z, Li L, Zhang D. Rapamycin promotes the survival and adipogenesis of ischemia-challenged adipose derived stem cells by improving autophagy. Cell Physiol Biochem. 2017;44(5):1762–1774.

63. Lee J-E, Schmidt H, Lai B, Ge K. Transcriptional and epigenomic regulation of adipogenesis. Mol Cell Biol. 2019;39(11): e00601–e00618.

64. de Sá PM, Richard AJ, Hang H, Stephens JM. Transcriptional Regulation of Adipogenesis. In: Prakash Y.S, editor. Comprehensive Physiology. Vol. 7. Hoboken (NJ): John Wiley & Sons, Inc; 2017. p. 635–674.

65. Huang K-T, Hsu L-W, Chen K-D, Kung C-P, Goto S, Chen C-L. Decreased PEDF expression promotes adipogenic differentiation through the up-regulation of cd36. Int J Mol Sci. 2018;19(12):3992.

66. Kim YW, Min HJ, Choi RJ, Lee DH, Cheon YW. Insulin promotes adipose-derived stem cell differentiation after fat grafting. Plast Reconstr Surg. 2018;142(4):927–938.

67. WANG CHAO, LI X, DANG H, LIU PING, ZHANG BO, XU FENG. Insulin-like growth factor 2 regulates the proliferation and differentiation of rat adipose-derived stromal cells via IGF-1 R and IR. Cytotherapy. 2019;21(6):619–630.

68. Doornaert M, Colle J, De Maere E, Declercq H, Blondeel P. Autologous fat grafting: latest insights. Ann Med Surg. 2019; 37:47–53.

69. Shuai Y, Yang R, Mu R, Yu Y, Rong L, Jin L. MiR-199a-3p mediates the adipogenic differentiation of bone marrow-derived mesenchymal stem cells by regulating KDM6A/WNT signaling. Life Sci. 2019;220:84–91.

70. Xing W, Mu D, Wang Q, Fu S, Xin M, Luan J. Improvement of fat graft survival with autologous bone marrow aspirate and bone marrow concentrate. Plast Reconstr Surg. 2016;137(4): 676e–686e.

71. Jianhui Z, Chenggang Y, Binglun L, Yan H, Li Y, Xianjie M, Yingjun S, Shuzhong G. Autologous fat graft and bone marrow–derived mesenchymal stem cells assisted fat graft for treatment of Parry-Romberg syndrome. Ann Plast Surg. 2014;73(Suppl 1):S99–S103.

72. Huang H, Feng S, Zhang W, Li W, Xu P, Wang X, Ai A. Bone marrow mesenchymal stem cell-derived extracellular vesicles improve the survival of transplanted fat grafts. Mol Med Rep. 2017;16(3):3069–3078.

73. Mashiko T, Yoshimura K. How does fat survive and remodel after grafting? Clin Plast Surg. 2015;42(2):181–190.

74. Harris WM, Plastini M, Kappy N, Ortiz T, Chang S, Brown S, Carpenter JP, Zhang P. Endothelial differentiated adipose-derived stem cells improvement of survival and neovascularization in fat transplantation. Aesthetic Surg J. 2019;39(2):220–232.

75. Lauvrud AT, Kelk P, Wiberg M, Kingham PJ. Characterization of human adipose tissue-derived stem cells with enhanced angiogenic and adipogenic properties. J Tissue Eng Regen Med. 2017;11(9):2490–2502.

76. Choi JW, Kim S-C, Park E-J, Lee J-A, Jeong WS. Positive effect of incubated adipose-derived mesenchymal stem cells on micro-fat graft survival. J Craniofac Surg. 2018;29(1):243–247.

77. Yuan Y, Gao J, Liu L, Lu F. Role of adipose-derived stem cells in enhancing angiogenesis early after aspirated fat transplantation: induction or differentiation? Cell Biol Int. 2013;37(6):547–550.

78. YUAN Y, GAO J, LU F. Effect of exogenous adipose-derived stem cells in the early stages following free fat transplantation. Exp Ther Med. 2015;10(3):1052–1058.

79. Chen J, Gu Z, Wu M, Yang Y, Zhang J, Ou J, Zuo Z, Wang J, Chen Y. C-reactive protein can upregulate VEGF expression to promote ADSC-induced angiogenesis by activating HIF-1α via CD64/PI3k/Akt and MAPK/ERK signaling pathways. Stem Cell Res Ther. 2016;7(1):114.

80. Xiong X, Sun Y, Wang X. HIF1A/miR-20a-5p/TGFβ1 axis modulates adipose-derived stem cells in a paracrine manner to affect the angiogenesis of human dermal microvascular endothelial cells. J Cell Physiol. 2020;235(3):2091–2101.

81. Mou S, Zhou M, Li Y, Wang J, Yuan Q, Xiao P, Sun J, Wang Z. Extracellular vesicles from human adipose-derived stem cells for the improvement of angiogenesis and fat-grafting application. Plast Reconstr Surg. 2019;144(4):869–880.

82. Han Y, Bai Y, Yan X, Ren J, Zeng Q, Li X, Pei X, Han Y. Co-transplantation of exosomes derived from hypoxia-preconditioned adipose mesenchymal stem cells promotes neo-vascularization and graft survival in fat grafting. Biochem Biophys Res Commun. 2018;497(1):305–312.

83. Hoseini SJ, Ghazavi H, Forouzanfar F, Mashkani B, Ghorbani A, Mahdipour E, Ghasemi F, Sadeghnia HR, Ghayour-Mobarhan M. Fibroblast growth factor 1-transfected adipose-derived mesenchymal stem cells promote angiogenic proliferation. DNA Cell Biol. 2017;36(5):401–412.

84. Zhou S, Chang Q, Lu F, Xing M.Injectable mussel-inspired immobilization of platelet-rich plasma on microspheres bridging adipose micro-tissues to improve autologous fat transplantation by controlling release of PDGF and VEGF, angiogenesis, stem cell migration. Adv Healthc Mater. 2017;6(22):1700131.

85. Chung NN, Ransom RC, Blackshear CP, Irizarry DM, Yen D, Momeni A, Lee GK, Nguyen DH, Longaker MT, Wan DC. Fat grafting into younger recipients improves volume retention in an animal model. Plast Reconstr Surg. 2019;143(4):1067–1075.

86. Cai J, Feng J, Liu K, Zhou S, Lu F. Early macrophage infiltration improves the fat graft survival by inducing angiogenesis and hematopoietic stem cell recruitment. Plast Reconstr Surg. 2017;79(1):146–158.

87. Liu K, Cai J, Li H, Feng J, Feng C, Lu F. The disturbed function of neutrophils at the early stage of fat grafting impairs long-term fat graft retention. Plast Reconstr Surg. 2018;142(5):1229–1238.

88. Bouglé A, Rocheteau P, Hivelin M, Haroche A, Briand D, Tremolada C, Mantz J, Chrétien F. Micro-fragmented fat injection reduces sepsis-induced acute inflammatory response in a mouse model. Br J Anaesth. 2018;121(6):1249–1259.

89. Krastev TK, Beugels J, Hommes J, Piatkowski A, Mathijssen I, van der Hulst R. Efficacy and safety of autologous fat transfer in facial reconstructive surgery. JAMA Facial Plast Surg. 2018;20(5):351.