Chapter 3

The ASC: Critical Participants in Paracrine-Mediated Tissue Health and Function

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/55545

1. Introduction

1.1. The adipose-derived stem cell — A pluripotent adult stem cell?

In 2001, the journal Tissue Engineering published an article describing the isolation of a population of putative multipotent stem cells from adipose tissue termed Processed Lipoaspirate Cells or PLA cells [1]. Based on isolation methods designed for the harvest of adherent, fibroblastic cells from the adipose stroma capable of adipogenic differentiation in vitro [2], this work by Zuk et al. described the differentiation of their PLA cells toward multiple mesodermal lineages, including fat, bone and cartilage. This ground-breaking article has since been followed by over 3500 studies published and available through PubMed, describing the differentiation capacity of ASCs in a variety of in vitro and in vivo model systems. Early works continued the characterization of PLA cells – now termed ASCs for Adipose-derived Stem Cells - identifying a unique CD "signature" for these cells [3]-[8] and studying their mesodermal differentiation capacity at a molecular and biochemical level [8]. Subsequent studies have since confirmed the ASC's mesodermal differentiation capacity in vitro reporting osteogenic, adipogenic, chondrogenic and skeletal myogenic capacities [9]-[20]. These works have since been expanded into in vivo translational models using a variety of animal systems for bone formation [21]-[25], cartilage [26]-[28], fat [29]-[32] and skeletal muscle [33]-[35]. In addition, recent years have presented some exciting results, expanding ASC potential to add smooth muscle [36], [37] and cardiac myogenesis [38], [39] to the growing list of ASC capacities.

With these increased capacities, it became natural to ask if the ASC possessed pluripotent potential and initial in vitro studies appeared to answer this question, reporting ectodermal [8], and endodermal differentiation [40], [41]. However, the true test of these germ line potentials still lies in the in vivo model. Consistent with the in vitro studies, numerous in vivo

model systems have reported possible ectodermal and endodermal potentials, describing the repair of nervous and epithelial tissues [42], [43], together with hepatic and pancreatic regeneration [44]-[46]. With these in vivo results, combined with earlier in vitro analysis, it becomes easier to conclude that the ASC is an adult pluripotent stem cell population.

1.2. ASC-mediated tissue regeneration: Secretion of soluble factors

Despite the in vivo translational studies above suggesting that ASCs are capable of enhancing tissue healing and regeneration, many of these studies cannot confirm the direct differentiation of the ASC into a specific cell type. For example, while bone regeneration is observed upon implantation of ASCs, very few studies report the presence of the ASC within the newly formed bone. Whether this is an oversight by the research team or an indication that the ASC does not directly form part of the new tissue is unclear. It is entirely possible that the ASC does not directly differentiate into the desired regenerating tissue, but simply directs tissue formation "from the sidelines". Tissue development and healing is incredibly complex and the role of paracrine signaling is still not entirely understood. Therefore, it is possible that ASCs may be intimately involved in tissue regeneration and health through their ability to mediate the host's regenerative capacity using paracrine signaling.

Two arguments can be made in support of this theory. First, in many translational models, it does not appear that the ASC has any difficulty in surviving within the transplantation region for extended periods of time. In addition, the range of tissues capable of engrafting ASCs appears to be quite broad. Initial studies by Nolta and researchers show that systemic administration of human ASCs is followed by multi-organ engraftment in nude mice [47]. In support of this, human ASCs administered via tail vein migrate and home efficiently to multiple tissues (epithelial and endothelial) in irradiated mice [48], [49]. The specific migration of ASCs to injured tissues has also been shown by the Longaker group, who confirm the presence of ASCs specifically in parietal bone defects and their persistence as the defect heals [50]. Second, stem cells like bone marrow MSCs and ASCs are known to secrete numerous factors and cytokines, including VEGF, HGF, NGF, BDNF and multiple interleukins [49], [51]. In fact, Salgado's article calls these factors the "secretome" of ASCs. This secretome may have powerful paracrine effects on the health, repair and function of a tissue and has resulted in an exciting, new theory that proposes the ASC as a mediator of tissue regeneration through the secretion of specific soluble factors. In this regard, the ASC could be used in an incredibly broad range of applications. However, the most popular are reviewed below.

2. The use of ASCs in transplantation — Immunomodulatory and anti-inflammatory actions

Successful transplantation is reliant upon tolerance by the host's immune system. In 2000, human MSCs were transplanted into immunocompetent sheep without significant rejection [52], suggesting that adult stem cells might survive in a xenogeneic environment. Subsequent work with MSCs has described their ability to immunosuppress mixed lymphocyte reactions

http://dx.doi.org/10.5772/55545

and to suppress stimulated T cell proliferation [53]-[55]. MSCs are also known to inhibit cytotoxic T lymphocyte toxicity [56], [57] and inhibit B cell proliferation by altering the G0/G1 transition [58]. Likewise ASC-mediated immunosuppression has been confirmed through a series of elegant in vitro experiments that describe the suppression of mixed lymphocyte reactions and/or proliferation of key immune cells like the T cell [59]-[63]. Immunosuppression has also been observed in a variety of in vivo model systems (Table 1). For example, reduced inflammatory infiltration and airspace enlargement results from the systemic administration of human ASCs to murine models of emphysema [64]. Moreover, the ASCs are capable of rescuing the suppressive effects of cigarette smoke on bone marrow hematopoietic progenitor function [64]. Experimental autoimmune hearing loss can be treated in mice through the systemic infusion of human ASCs, resulting in protection of hair cells possibly through the production of the anti-inflammatory cytokine IL10 by splenocytes [65] and decreasing the proliferation of antigen-specific Th1 and Th17 cells. Similar immunosuppression and amelioration of disease is reported upon injection of ASCs in models of rheumatoid arthritis [66] and IgA nephropathy [67], resulting in decreased inflammatory markers and Th1 cytokine activity, together with the generation of regulatory T cells capable of suppressing T cell responses. Finally significant anti-inflammatory responses are observed upon the transplantation of allogeneic murine ASCs into dystrophin-deficient mice, decreasing markers of oxidative stress and inflammation, including TNFα and IL6, decreasing production of CD3+ T cells, and enhancing the synthesis of anti-inflammatory IL4 and IL10 [68]. While these studies are supportive of the role for ASCs in modulating immune responses, what remains unknown is the mechanism. One theory proposes that cell-cell contact is required [61]. However, others dispute this finding, suggesting that it is the secretion of soluble factors by the ASC that mediates the eventual reaction by the host's immune system [69]. In support of this, inhibition of prostaglandin E2 production in ASCs by indomethacin can abolish the immunosuppressive properties of ASCs. Alternatively, neutralizing leukemia inhibitory factor has had similar effects [70]. Finally, there are those that suggest a role for IL-6 [55].

The immunosuppressive properties of ASCs may make it possible to use more xenogeneic transplantation model systems without the fear of significant immune reactions in animal hosts. Such models would allow for a more direct study of human ASCs in vivo, thus allowing researchers to more accurately predict what these cells could do clinically. An excellent review of these models can be found in a recent article by Lin et al. [81]. In this article, they present a detailed table outlining many of the recent xenogeneic model systems, such as one by Paul and colleagues [82], who perform a xenogeneic transplantation of human ASCs into myocardial infarcts produced in immunocompetent rats. Histology confirms human ASCs in the infarct region after 6 weeks, with no detectable inflammatory reaction even in the absence of immunosuppressive action. Furthermore, these animals show improvement of cardiac function and reduced infarct size, together with significant improvement in myocardial anti-inflammatory cytokine levels. The success of such xenogeneic transplantation models may be explained, in part, by the immunogenic profile of the ASC. Immunophenotyping of ASCs has not only provided researchers with a CD antigen profile but has confirmed the absence of the HLA-DR antigen on the ASC surface. Divided into classes such as HLA-A, B and C (or MHC

Author and Year (Reference)	ASC type	Disease Model	Inflammatory/Immunosuppressive action
Pinheiro et al. 2012 [68]	human	murine dystrophy	decreased CD3+ve T cells, increased IL-4, IL-10 synthesis
Payne et al. 2012 [71]	human	autoimmune demyelination – IL-4 overexpressing ASCs	increased T cell responses
Zhou et al. 2011 [65]	human	autoimmune hearing loss	secretion of IL-10, decreased proliferation of Th1, Th17 cells
Hyun et al. 2011[67],	mouse	IgA-induced nephropathy	decreased inflammatory markers, decreased Th1 activity
Schweitzer et al. 2011 [64]	human, mouse	emphysema	decreased inflammatory infiltration
Lai 2011 et al. [72]	human	systemic lupus erythamatosis	decreased Th17 production, decrease IL-17 synthesis
Zhou 2011 et al. [66]	human	rheumatoid arthritis	decreased Th1, Th17 proliferation/expansion, increased IL10 synthesis
Kuo 2011 et al. [73]	rat	hind limb allotransplantion	increased Treg proliferation
Gonzalez-Rey et al. 2010 [74], Gonzalez et al. 2009 [75]	human	rheumatoid arthritis	inhibition of CD4+ T cell proliferation, increase in IL-10 producing T cells and monocytes, stimulation of Treg cell development
Cho et al. 2010 [76]	mouse	airway allergic disease	decreased airway inflammation, shift from a Th2 to a Th1-biased immune reponse
Gonzalez-Rey et al. 2009 [77], Gonzalez et al. 2009 [78]	human	experimental colitis	decrease in Th1-driven inflammation, decrease inflammatory cytokines, increased IL-10 activity
Kim et al. 2007 [79]	human	hemorrhagic stroke	decreased brain inflammation markers
Wan et al. 2008 [59]	rat	orthotopic liver transplant	increased IL-2 and IL-10 synthesis
Constatin et al. 2009 [80]	mouse	autoimmune encephalolyelitis (multiple sclerosis)	increased Th2-type shift in cytokine production[80]

Table 1. Immunosuppressive action of ASCs

class I) and HLA-DP, DM and DR (or MHC class II), HLA receptors display proteins on the cell surface for immune surveillance. Of particular interest is the HLA/MHC class II protein, which is found on the surface of antigen-presenting cells and plays critical roles in immunotolerance and transplantation (for reviews see [83], [84]). The absence of this class of HLA protein may allow the ASC to evade the host's immune surveillance machinery. Of additional interest is a recent study by DelaRosa et al. [85], who note that human ASCs have lower susceptibility to natural killer (NK) cell-mediated lysis in comparison to bone marrow MSCs.

This finding may be part of the reason for xenogeneic tolerance of ASCs in that NK-ASC crosstalk does not result in immediate recognition. Continued research in this area is sure to expand the possible uses of ASCs in translational model systems.

3. Vascularization by ASCs in tissue repair

Tissue repair and regeneration is reliant upon vascularization. Newly formed tissues must have sufficient blood flow to maintain their health and support their growth. Early in vitro studies with ASCs suggest the capacity to differentiate into endothelial cells and to form vessel-like structures. For example, using simple in vitro induction conditions, ASCs express typical markers of endothelial cells, such as von Willebrand Factor (vWF) and function as endothelial cells, taking up acetylated LDL and forming tubular structures on Matrigel substrates [40], [41], [86]. Tubule formation, LDL uptake and CD31 expression by ASCs are also found upon in vitro exposure to shear stress [87], [88]. Such evidence provides strong support for the use of ASCs in the induction of vessel formation and some have attempted to isolate the specific ASC subpopulation that might be responsible for endothelial differentiation. For example, Wosnitza et al. postulate that a population of CD31-ve, S100+ve ASCs are capable of endothelial differentiation [89], while CD34-ve ASCs have been observed to undergo differentiation by others [90].

Author and Year (Reference)	ASC type	Secreted Factor
Ribeiro et al. 2012 [91]	human	VEGF, HGF, bFGF, NGF, SCF
li et al. 2012 [92]	human	VEGF, bFGF, SDF1α
Kim et al. 2011 [93]	human	VEGF
Lu et al. 2011 [94]	human	VEGF, HGF, BDNF, NGF
Liu et al. 2011 [95]	rat	HGF
Nie et al. 2011 [96]	rat	VEGF, HGF, bFGF
Salgado et al. 2010 [49]	human	VEGF, HGF, BDNF
Zhu et al. 2010 [97]	human	VEGF
Grewal et al. 2009 [98]	human	VEGF
Rubina et al. 2009 [99]	mouse	VEGF, HGF, bFGF, PDGFB, TGFb
Park et al. 2008 [100]	human	VEGF, HGF, PDGF
Prichard et al. 2008 [101]	rat	VEGF
Kilroy et al. 2007 [102]	human	HGF
Wang et al. 2006 [103]	human	VEGF, HGF, IGF-1
Cao et al. 2005 [41]	human	VEGF, HGF, bFGF, KGF, TGFβ
Rehman et al. 2004 [104]	human	VEGF

Table 2. Growth factor secretion by ASCs

However, the efficacy of ASCs in tissue repair may not be entirely due to their direct differentiation into endothelial lineages, but also to their secretion of paracrine factors capable of

increasing vascularization. In support of this, co-culture of ASCs with postnatal cardiomyocytes results in the formation of stable, branching CD31+ve vessel-like structures that disassemble in the absence of ASCs [99]. Similarly, ASC-conditioned media can induce the formation of vessel-like tubules within Matrigel [105]. More recently, while rat ASCs express Flt-1, CD31 and vascular endothelial cadherin, when injected into a wire injury model in the rat femoral artery, induction of endothelial repair occurs without any observable differentiation of these ASCs into endothelial cells [106]– a finding that can be explained if repair is driven through the production of soluble factors. In the hopes of identifying what angiogenic factors improve a tissue's vasculature, numerous studies have characterized the secretion of growth factors by ASCs (Table 2). Of all of these factors, perhaps the most commonly reported is VEGF, with secretion of this factor being reported under normal culture conditions [98], hypoxic conditions [104] in models of wound healing [96], [107] and cell-assisted lipotransfer [97]. The ability of VEGF to stimulate neoangiogenesis is well known [108]-[110]. Consistent with this, conditioned medium from ASCs, maintained under hypoxic culture conditions in order to increase production of HGF, VEGF and TGFβ, has been found to increase endothelial cell (EC) growth and reduce their apoptosis [104]. In addition, VEGF secretion by ASCs is significantly upregulated in vitro upon metabolic induction of ischemia [111]. However, the role of other secreted factors cannot be ruled out as suppression of HGF production by ASCs through RNA interference significantly impairs ischemic tissue revascularization [112] and SDF-1α from ASCs has been identified as being involved in myocardial vascularization [92]

3.1. Ischemia/ischemia-reperfusion injury

Today, there are several model systems that study the paracrine-mediated vascularization potential of ASCs but some of the most common are: ischemia and ischemia-reperfusion (IR) injuries, wound healing and cardiac infarct treatment. Enhanced angiogenesis within ischemic limbs has been reported following treatment with freshly isolated ASCs (i.e. the stromal vascular fraction) and vessels derived from these cells confirmed [113]. However, the use of such a heterogenous population makes it difficult to confirm direct ASC involvement. Fortunately, there have been numerous studies describing the beneficial use of cultured/purified ASCs in the treatment of ischemia [86], [90], [93], [114]-[117]. Consistent with paracrine action, improved vascularization within ischemic limbs has been associated with increased levels of plasma VEGF [93]. In addition, human ASCs cultured in vitro as spheroids improve neovascularization and limb survival when compared to the implantation of dissociated ASCs – a finding thought to be due to the induction of vascular factors, like HGF, VEGF and bFGF, by the hypoxic conditions of the spheroid [118]. In support of this, decreases in the ability of ASCs to induce reperfusion in ischemic hindlimbs are observed if secretion of HGF by the ASC is inhibited [112]. However, the role of the ASC in angiogenesis may not be restricted to their secretion of established angiogenic factors. Transplantation of ASCs transfected with siRNA to either MMP3 or MMP9 to ischemic hind-limbs results in lower blood flow recovery and higher tissue injury [119], suggesting that ASCs may also promote angiogenesis through their secretion of matrix-remodelling enzymes.

http://dx.doi.org/10.5772/55545

Whereas prolonged ischemia can cause significant tissue damage, there is evidence now that the reperfusion period is also associated with injury, amplified by the production of reactive oxygen species and inflammatory cascades [120]. Events such as these are a major obstacle to successful tissue transplantation. However, the ASC may ameliorate IR injury through its secretion of pro-angiogenic factors, thus increasing the density of developing capillaries within the reperfused tissue. Consistent with this, a significant increase in pro-angiogenic factors can be confirmed in IR skin flap models treated with ASCs [121]. Long-lasting improvement in cardiac function with increased angiogenesis and vasculogenesis can also be observed in IR in minipigs treated with a trans-endocardial injection of ASCs [122] and a higher number of CD31+ve and vWF+ve cells have been found in models of lung IR followed by ASC injection [123]. While the finding that ASCs can form vessel-like structures in Matrigel in vitro and re-endothelialize carotid injuries in vivo [87], [124] may suggest that the observed angiogenesis is due to differentiation by ASCs, the failure to observe significant ASC engraftment in IR models [122] again suggests that the role of ASCs may be paracrine in nature.

In addition to stimulating angiogenesis, the ASC may also lessen the damaging effects of IR through paracrine secretion of a combination of anti-inflammatory and anti-oxidant factors. The production of oxidative toxins such as free radicals and reactive oxygen species in ischemia and IR is well-established [125]-[128]. The synthesis of enzymatic anti-oxidants, such as superoxide dismutase and glutathione peroxidase, not only can be detected by proteomic analysis in ASC-conditioned media, but this media is able to protect dermal fibroblasts from oxidative damage [129]. Therefore, the ASC may be an excellent candidate for protection against oxidative damage. In support of this, Chen and co-workers, using a model of kidney IR treated with either conditioned medium from ASCs or direct injection of ASCs during reperfusion, find increased clearance of creatinine and urea from blood plasma in ASC/IR groups together with higher levels of the anti-oxidant markers NAD(P)H quinine oxidoreductase, heme-oxygenase 1/HO-1, glutathione peroxidase and glutathione reductase [130]. Increased anti-oxidant marker levels (i.e. NAD(P)H quinine oxidoreductase and HO-1) have also been reported, together with increased eNOS expression and decreased hepatic oxidative stress versus controls upon multiple injections of ASCs in hepatic IR models [131]. These anti-oxidant actions by ASCs are not only likely to protect the reperfused tissue from oxidative damage but may also protect the ASC itself. A recent study by Suga and colleagues suggests that resident ASCs are resistant to ischemia-mediated damage, surviving within ischemic adipose grafts [132]. Moreover, this work specifically postulates that the actions of these resident ASCs may be responsible for the observed increases in vascular density and the number of new adipocytes over time. Therefore, ASCs may be resistant to the toxic environment of ischemic tissues and may retain their functional capacities, thus being able to either differentiate or secrete paracrine factors for critical for angiogenesis.

3.2. Wound healing

Paracrine action is also likely to play a significant role in the beneficial effects of ASCs in wound healing models. ASCs isolated from debrided skin are capable of producing an epithelial layer when seeded into collagen gels, together with a dermis when seeded fibrin gels are co-cultured

with ASC/collagen/epithelial constructs, suggesting that the ASC would be an excellent cell source for healing skin wounds [133]. In support of this, increased collagen density has been reported in full-thickness rat skin grafts injected with ASCs [134] and Lim et al. [135] note improved wound healing rates upon implantation of ASCs. These wound healing rates are significantly higher than in controls treated with ASC extracts, suggesting that production of paracrine factors by viable ASCs are necessary in order to direct the formation of new tissue within the wound. In vitro culture of immortalized keratinocytes or dermal fibroblasts with ASC-conditioned medium results in increased proliferation of these cells, in addition to increased transcription and production of collagen type I, suggesting that secreted ASC-derived factors may ultimately influence keratinocyte-mediated healing in skin grafts [136], [137]. Finally, Jung and colleagues have reported that conditioned medium from ASCs can increase CNI, CNIII and hyaluronic acid synthesis by human dermal fibroblasts and that neutralizing antibodies to TGFβ1 can abolish this effect [138]. However, it is equally likely that improved wound-healing using ASCs is due to their secretion of angiogenic factors, thus improving healing through augmentation of vascularization. As proponents of this theory, Reichenberger et al. [139] and Gao et al. [107] report higher blood flow and skin flap survival, respectively when the flaps are combined with ASCs. In addition, Gao and colleagues report increased capillary density, together with increased expression of VEGF within the dermis in the ASC-treated groups. In support of this, increased VEGF expression and microvascular density is also measured in ASC-treated rat skin grafts [134]. Interestingly, recent studies suggest that AKT/c-myc signaling pathways may mediate increased VEGF secretion in ASCs as injection of constitutively active AKT/v-myc-expressing ASCs promote better wound healing compared to normal controls [140]. How exactly the ASC promotes wound healing is likely to be a combination of increased tissue healing and vascularization as directed by their secretion of specific paracrine factors. In support of this, GFP-labelled ASCs not only secrete the angiogenic factors VEGF, HGF and bFGF in vivo, but co-stain with keratin and CD31 in excisional wound healing models in normal and diabetic rats, possibly undergoing both epithelial and endothelial differentiation [96]. Similar differentiation by human ASCs, implanted into skin wounds via silk/chitosan scaffolds, has also been reported by Altman and colleagues [141]. Therefore, the successful use of ASCs in wound healing models may be due to their paracrine action in promoting angiogenesis by the host and their autocrine action in promoting differentiation in themselves.

3.3. Infarct treatment

In a 2007 study by Fotuhi, freshly isolated ASCs injected into porcine transmural infarcts were shown not to cause arrhythmia, bradycardia or conduction block. Moreover, these ASC-treated hearts required extra-stimuli to induce an arrhythmia, suggesting that ASCs could be used in the treatment of cardiac infarcts [142]. With in vitro studies confirming the cardiomyogenic potential of these stem cells, infarct treatment could be mediated through the differentiation of ASCs into cardiomyocytes. However, there is a debate on whether the ASC contributes directly to cardiac muscle regeneration or supports this event through the production of angiogenic growth factors and cytokines. An example of this debate can be seen in the 2007 article by Zhang et al. [143]. Rabbit ASCs injected into transmural infarcts in hearts three wks

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after occlusion decrease transmural scar and improve left ventricle ejection fraction (LVEF), end-diastolic pressure and myocardial performance relative to saline controls, with ASCs pre-induced with 5-azacytidine for 24 hours giving slightly better results versus untreated controls. When the infarct region is examined histologically, the ASCs form islands of cardiac tissue in and around the scar. However, all infarcts treated with ASCs also show greater capillary density, with the ASCs also differentiating into endothelial cells. Increased capillary densities/angiogenesis have previously been reported using bone marrow mononuclear cells and endothelial progenitors and MSCs are known to cause improvement in cardiac function by incorporating into newly formed capillaries and releasing angiogenic factors [144]. Similar events may also be induced by ASCs. In support of this, mouse ASCs injected into murine infarcts take up residence in the infarct area, with EKGs showing stability of LVEF [145]. Murine ASCs [146] or rat ASCs [147] transplanted into rat infarcts result in significant improvement in heart function and tissue viability. Human ASCs not only increase peri-infarct capillary density in rat infarcts but increase numbers of nerve sprouts [148]. Finally, while Beitnes and co-workers show significant improvement in LVEF, smaller infarct sizes and increased vascularization when human ASCs are injected into infarcts in nude rats, they specifically observe an absence of ASC engraftment [149]. However, it is important to note that ASC engraftment was examined in this study 4 weeks post-transplant. It is possible that the long-term beneficial effects of ASCs on infarct treatment can result from short-term engraftment. In support of this, while transdifferentiation of human ASCs into cardiomyocytes or endothelial cells is also not observed in rat cardiac infarcts, the expression of VEGF, bFGF and SDF-1α can be confirmed in these hearts within the first few days of transplant and improved heart function and vascular density is ultimately observed [92]. Therefore, long-term survival of ASCs within the myocardium may not be necessary for their beneficial effects on cardiac function to be realized. Such a possibility would be extremely exciting if this treatment modality is translated into the clinic.

3.4. Other vascularization systems

In addition to wound healing, infarct treatment and ischemia-reperfusion, there are numerous other vascularization systems that might benefit from the putative angiogenic action of ASCs. Hemodynamic abnormalities may be reversed with the treatment of pulmonary arterial hypertension with ASCs through their augmented expression of HGF for angiogenesis and increased number of small pulmonary arteries [95]. Small-for-size liver injury may be treated through their secretion of VEGF. Inhibition of VEGF secretion by ASCs through RNA interference (RNAi) does not prevent apoptosis of liver sinusoidal endothelial cells in vitro and when cells are transplanted syngeneically results in significant disturbances to graft microcirculation, serum liver functional parameters and graft survival [150]. Finally, at the cosmetic level, cell-assisted lipotransfer fat grafts survive at higher levels, are 35% larger and show increased neoangigogenesis when compared to grafts transplanted without isolated ASCs [151].

4. Neuroprotection by ASCs — Demyelination, stroke, spinal cord injury

Early translational studies do suggest that ASCs can be safely administered to nervous tissue injuries and that functional improvement is noted. Transplanted ASCs have been reported to improve functional deficits following middle cerebral occlusion or ischemic stroke [152]-[154], spinal cord contusion injury [155] and peripheral nerve gaps [156], [157]. Histologic analysis of these injury sites has suggested that ASC differentiation into neurons and/or glial cells may play a role in the functional recovery, with transplanted cells staining positively for MAP2 [153], GFAP, Tuj-1 and an oligodendrocyte marker [155]. However, this functional improvement may be due to paracrine actions on the host more than ASC differentiation, as less then 1% of transplanted ASCs can be found within a spinal contusive injury model, with those remaining appearing to be oligodendrocytes [158]. In addition, extremely low levels ASC differentiation into mature neurons is noted in a model of cerebral cortex injury [159]. However, both of these studies note significant changes in the host tissue with Nakada et al. observing improvements in microvasculature and Zhang et al. measuring increases in host oligodendrocyte formation. Therefore, like wound healing and IR models, ASCs are likely to exert paracrine actions within nervous tissue.

In 2002, Zhao et al. suggested that functional recovery in ischemic brain injury was not due to MSC differentiation but to secreted paracrine factors that act on the host [160]. A similar hypothesis has been put forth by bone marrow MSC groups who have noted increased survival and differentiation of Tuj1+ve neurons and neuroblastoma cells in co-cultures [161] and increased neuronal viability and glial cell differentiation using MSC conditioned media [162]. Consistent with this, ASC/Matrigel constructs implanted into models of mice limb re-innervation stimulate the regeneration of nerves and induce axon growth, likely through the expression numerous neurotrophins [163]. Moreover, enhanced nerve fiber growth is observed if the ASCs are pre-induced toward the neural lineage thus enhancing their production of brain-derived neurotrophic factor (BNDF). BDNF secretion (together with nerve growth factor/NGF and glial cell-derived neurotrophic factor/GDNF) by ASCs pre-differentiated toward a Schwann Cell (SC) phenotype is thought to be the basis for axonal regeneration in sciatic nerve gap models - although these authors speculate that this regeneration is likely due to the neuroprotective function of these three neurotrophins [164]. In support of this, studies using ASC-conditioned media appeared to further strengthen this theory. Protection against cortical and hippocampal volume loss in rats can be achieved through the infusion of ASC-conditioned medium [165]. ASC-conditioned medium containing VEGF, BDNF and NGF is shown to have a protective effect against glutamate excitotoxicity on PC12 cells (a key factor implicated in stroke and neurodegenerative diseases) and increase PC12 viability [94]. Conditioned media from pre-differentiated ASCs infused over one week into a rat model of ischemic stroke 8 days after stroke induction increases the number of CD31+ve cells [166]. Finally, functional deficits in a model of middle cerebral artery occlusion can be dramatically improved using ASC transduced to overexpress BDNF [153].

While these neurotrophic factors may act to protect neurons, ASCs may also play roles in decreasing inflammation and gliosis (i.e. glial cell-mediated scar formation) – two critical

http://dx.doi.org/10.5772/55545

events that specifically affect healing in the both the central and peripheral nervous system. Systemic transplantation of human ASCs can attenuate cerebral degeneration in rats, reducing both brain atrophy and glial proliferation [79]. Rats implanted with ASC-derived SCs show significant locomotor function recovery compared with untreated ASCs and also reduction in gliosis [152]. Pre-differentiated canine ASCs in Matrigel scaffolds show better functional recovery and reduced fibrosis and inflammation when implanted into spinal cord injuries [167]. Decreased gliosis is also noted upon intrathecal administration of ASCs in a model of IR neuronal damage in rabbits – an event accompanied by increased expression of BDNF within the first 72 hours following ASCs administration [168]. Finally, a possible anti-inflammatory role for ASCs in sciatic nerve repair might be seen in a recent model describing possible immunosuppression of xenogeneic acellular nerve matrices combined with autologous ASCs [169]. Implantation of this construct does not result in host rejection, making it possible that peripheral nerves repair can be accomplished using commercial nerve matrices combined with the patient's own ASCs.

4.1. Controlled release from ASCs — ASCs as a cellular biopump

It is possible that the paracrine action of ASCs may be "fine-tuned" so that the ASC secretes a desired factor, hence turning the ASC into a "cellular biopump". This is not a recent concept as the engineering of numerous cell types to secrete a variety of factors has been reported in the literature for over a decade. In the field of stem cell research, bone marrow MSCs have been modified to secrete various factors, including BMP2 [170], [171], bFGF [172], IFN-β [173] and IL12 [174]. Similar to these studies, ASCs have been engineered for the delivery of BMP4 [175], BMP2 [176], [177], and BMP6 [178] in several bone regeneration models. Delivery of TGFβ2 by ASCs for the induction of chondrogenesis has been reported [179]. Adenovirally-mediated VEGF secretion by ASCs has been used to induce vascular growth in a bone defect model [180] and adipose tissue grafts [181]. Finally, as described above, BDNF delivery by transduced ASCs into a model of middle cerebral artery occlusion improves functional deficits when compared to control ASCs [153].

However, a more exciting idea might be in the engineering of ASCs in the treatment of disease. In 2007, ASCs engineered to express cytosine deaminase were found to decrease the growth of colon carcinoma cells [182]. ASCs have recently been described in the delivery of an oncolytic myxoma virus that will specifically target gliomas [183]. ASC viability is not impacted with transduction and successful cross-infection of gliomablastoma cells is observed upon 3D co-culture with glioblastoma cells, leading to their cell death. More importantly, rat survival is increased with this myxoma virus delivery, with the size of the gliomas significantly decreasing upon injection of transduced ASCs in comparison to non-transduced ASCs controls. Localization of ASCs and increased apoptosis within tumors has also been reported following intravenous or subcutaneous injection of ASCs engineered to express TRAIL, having no effect on the surrounding healthy tissue [184]. Finally, this approach may have far-reaching effects on autoimmune diseases through the delivery of interleukins and interferons. ASCs engineered to overexpress IL4 and administered at the time of T cell priming attenuate autoimmune encephalomyelitis and reduce peripheral T cell responses shifting the host pro-inflammatory

response to an anti-inflammatory one [71]. With the development of inducible viral systems, there is the possibility that the ASC cellular biopump could be controlled not only at the dose level through the number of cells delivered but at the temporal level, giving clinicians more precise control over their therapeutic regimen.

4.2. ASC uses in the clinic

In light of their differentiative capacity and paracrine actions, there is great interest in the use of ASCs within the clinic. As source of regenerative stem cells, the ASC may have no equal. Bone marrow aspirates yield on average $6x10^6$ nucleated cells per ml, of which, only 0.001 to 0.01% are thought to be stem cells [185], [186]. In comparison, approximately three-fold more cells can be obtained per gram adipose tissue [187] [188] with 10% of these cells thought to be stem cells [188], [189]. The abundance of ASCs within adipose tissue, combined with the relative ease of its harvest and isolation also makes the ASC a good choice for clinical work. Patient's could conceivably have their adipose tissue harvested relatively painlessly a few weeks prior to their procedure in a simple outpatient procedure, the ASCs isolated and expanded under good manufacturing protocols and then used for regenerative purposes. With the confirmed absence of HLA/MHC class II proteins and continuing xenogeneic animal models, the patient may not even need to use their own stem cells. Donated allogeneic ASC lines could be used in lieu of autologous cells without the fear of immunorejection or inflammatory complications. Such a situation might be perfect in the case of myocardial infarct treatment where a delay in treatment could have serious consequences.

The first published article using ASCs in a clinical setting was in 2004, in which freshly harvested SVF cells were combined with fibrin glue and used in the repair of a traumatic calvarial injury [190]. Three months after reconstruction, CT scans showed new bone formation within the injury. However, it is important to point out that the cells used in this study were not ASCs, purified through plastic adherence and culture time, but the SVF - a heterogenous mixture of ASCs, endothelial cells, pre-adipocytes, pericytes, fibroblasts and red blood cells. Therefore, it is difficult to attribute the observed healing to the action of the ASC itself. Since that time, other clinical studies using the SVF have been attempted [191] and a review by Casteilla et al. does an excellent job of summarizing these works [192]. It is worth noting that with the exception of some cysts and microcalcifications being observed upon breast reconstruction [193], the use of SVFs clinically has not resulted in any serious complications.

Because of its heterogeneity, clinical studies using purified ASCs have also been performed for the treatment of such disorders as critical limb ischemia and radiation therapy ([194], [195] – for a more comprehensive review, see [192]). Bone regeneration using ASCs has recently been reported in 2009 with the reconstruction of the maxilla being induced using ASC in combination with BMP2 [196]. Bony healing using BMPs has been documented in numerous translational animal models [197]-[201], making this clinical study an exciting addition to the ways bone regeneration and healing can be brought about in the clinic. However, many of these translational models fail to report the appropriate control – the amount of bone being formed just by the BMP itself. The first translational study to combine ASCs and a BMP (i.e. BMP2) failed to measure any significant improvement in bone formation when BMP2 and ASC+BMP2 groups were

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compared [197]. Since this study, others have appeared to suggest that BMP2 may not promote the in vivo osteogneic capacity of the ASC [202] but may, in fact, may have a deleterious effect on bone regeneration [203]. Since it is not possible to perform similarly controlled studies clinically, it remains unknown if the addition of ASCs to BMP-treated scaffolds provides any more advantage. However, It is worth noting that, as with the use of SVFs, administration of ASCs into human patients has not been associated with any adverse effects [204].

The first phase I clinical trials using ASCs were not conducted on bone formation or even fat grafting but in the healing of chronic fistulae in Crohn's disease [205]-[210]. In 2005, nine rectovaginal fistulae in four patients were treated with ASCs, purified and cultured for up to one month. Of the eight fistulae examined, six showed complete healing in 8 weeks [206]. These fistulae had previously failed to heal using conventional surgical treatments, thus justifying progression to more comprehensive phase II trials. In 2009, a larger phase II trial using patients with and without Crohn's fistulae were treated with ASCs [211]. As seen with their earlier clinical trial, the majority of Crohn's and non-Crohn's fistulae were healed completely using ASCs in comparison to controls. Currently, there are three phase II clinical trials recruiting for the use of ASCs in Crohn's disease fistulae (Clinicaltrials identifiers: NCT01011244, NCT01157650, NCT00999115, http://clinicaltrials.gov/ct2/results?term=adipose+derived +cells), in addition to one phase III trial (NCT00475410) recently completed [212].

One of the reasons ASCs are considered in the treatment of Crohn's disease is their ability to suppress inflammation. This review includes numerous examples of how the ASC may be capable of suppressing the immune system and recent clinical trials have attempted to take direct advantage of this quality. The treatment of multiple sclerosis (MS) with SVFs, containing ASCs, has been described by Riordan and colleagues in 2009, with the 3 enrolled patients showing improvement in numerous functional categories including balance and coordination [213]. The use of culture expanded ASCs in autoimmune diseases like hearing loss, MS and rheumatoid arthritis was recently discussed in 2011 [214]. Prior to this, ASCs have been proposed as a viable therapy for suppression of graft vs. host disease (GVHD) [215]-[218]. Each of these studies report favorable functional outcomes and propose ASCs, or their SVF counterpart, for the treatment of immune system disorders.

The most obvious application of the ASC clinically should be in breast reconstruction. In the lab, the combination of ASC-containing SVFs with fat grafts through a protocol called cell-assisted lipotransfer has enjoyed success [151]. Clinically, treatment of facial lipoatrophy has been reported [219] and two recent trials overseas has suggested that the ASCs within the SVF are capable of increasing breast volume and improving contour 6 months post-surgery [193], [220]. However, the use of ASCs in breast reconstruction is being pursued carefully in light of recent findings that link stem cells to cancer. Bone marrow MSCs have been found to increase proliferation of breast cancer cell lines [221] and subcutaneous injection of MSCs with tumor cells can favor their growth [222]. Similar to this, ASCs can increase tumorigenesis of established breast cancer lines [223]. In this study, ASCs not only promote the growth of metastatic pleural effusion cells both in vitro and in vivo but the ASC also secretes adipsin and leptin – both of which are known to promote breast cancer growth [224]. Additional work in MSCs has documented their ability to secrete large amounts of IL-6 and the corresponding increase in

the growth of estrogen receptor alpha-positive cell lines [225]. Increased expression of IL4 and IL10 have also been reported by ASCs isolated from breast cancer tissue [226], leading many to speculate that the ASC may be capable of altering the immune environment within the breast, resulting in the "protection" of the cancerous cells. Such a possibility could have far-reaching effects in the development of breast cancer and in its possible reoccurrence if ASCs are used in reconstruction. However, it is encouraging to find that cultured ASCs are resistant to the chemotherapies cisplatin, vincristine or comptothecin and that they still retain their stem cell characteristics [227]. Such findings could make it possible for a more natural reconstruction of the breast if ASCs are found not to contribute to the cancer itself.

4.3. "Paracrines gone wild" — ASCs and adipose disorders

With the proposed paracrine function of ASCs now well accepted, a re-examination of certain disorders and how the ASC might play a role might now be in order. The most obvious of these disorders would be obesity. However, studying the ASC might allow more information into lesser known dysfunctions such as lipedema and rare adipose disorders (RADs) like Dercum's and Madelung's disease. Normal fat has been described as having an anti-inflammatory milieu with adipocytes storing lipid, regulating energy metabolism, and, together with resident macrophages, secreting anti-inflammatory mediators such as IL-10 and adiponectin to protect against the possible development of inflammation-driven obesity [228]-[230]. However, with chronic nutrient overload, existing adipocytes increase their fat storage to become hypertrophic and resident pre-adipocytes (or ASCs) are thought to undergo increased differentiation to increase adipocyte number (i.e. hyperplasia). The hypertrophic adipocytes increase their secretion of "adipokines" - soluble factors known to affect angiogenesis and inflammation [231], [232]. Specifically, these adipocytes shift their adipokine production from anti-inflammatory to inflammatory, producing a series of feedback cascades that ultimately manifests in obesity [232].

Obesity has been recognized since the 1950s as a chronic state of low-level inflammation associated with excess accumulation of adipose tissue [233]. This inflammation is now thought to be a complex response to cellular events, such as hypoxia and oxidative stresses within the adipocyte. Figure 1 outlines the possible interacting events underlying obesity starting with the creation of hypertrophic adipocytes. These adipocytes become too large to be adequately supplied by the existing vasculature in the adipose depot, resulting in localized areas of hypoxia. This hypoxic state induces the production of numerous pro-inflammatory adipokines (e.g. IL1Rα, IL6, IL8, TNFα, MCP-1, leptin) and decreases the secretion of several key anti-inflammatory factors (e.g. IL10, adiponectin). Excellent reviews on these adipokines in obesity can be found in Fain et al. 2010 and Balistreri 2010. In these hypertrophic adipocytes, hypoxia is thought to induce oxidative stress [234], [235]. Oxidative stress is defined as an imbalance in the levels of reaction oxygen species (ROS) relative to the tissue's antioxidant capacity, resulting in the accumulation of oxidative products such as superoxide and hydroxyl radicals, reactive nitrogen species (RNS) and hydrogen peroxide [236]. Excess nutrients and hypertrophic adipocytes can produce ROS through: the nicotinamide dinucleotide phosphate oxidase (NOX) system [237], incomplete mitochondrial respiration due to excess free fatty acids [238]

and endoplasmic reticulum (ER) stress due to excess lipid storage [239]. Both mitochondrial and ER dysfunction have been demonstrated to increase the secretion of inflammatory adipokines [239], [240] and numerous studies in obesity models and obese subjects now exist linking hypoxia, oxidative stress and inflammation (reviewed in [236]). Concomitant with the development of hypertrophic adipocytes, there is a shift within the adipose tissue from M2 macrophages, found in normal adipose tissue, to a more pro-inflammatory M1 macrophage subset [241]-[243]. This shift is likely, in part, a consequence of the production of pro-inflammatory adipokines by adipocytes – such as MCP-1, but this infiltration is also likely to be due to the death of these adipocytes [244]. Consistent with this, "crown-like" structures of macrophages are known to be associated with necrotic adipocytes in obese murine adipose tissue [242]. These macrophages may directly contribute to the production of inflammatory agents within obese adipose tissue [245]. However, they may also augment adipokine production by the adipocyte through possible cross-talk mechanisms. While these mechanisms are unclear at this point, there are many who postulate that adipocyte-macrophage interaction is the key factor in inflammation and resulting obesity [230], [246], [247].

Author & Year (Reference)	Secreted factor
Blaber et al. 2012 [267]	IFNγ, IL8, IL9, IL12, IL17, TNFα
Hsiao et al. 2012 [268]	IL6, IL8, MCP-1, MCSF, RANTES
Bhang et al. 2011 [118]	HIF1α
Salgado et al. 2010 49	TNFα, IL6, IL8
Banas et al. 2008 [269]	IL6, IL8, IL1Rα, MCP-1, GMCSF
Kilroy et al. 2007[102]	IL6, IL8, TNFα, MCSF, GMSCF

MCSF – macrophage colony stimulating factor

GMCSF – granulocyte-macrophage colony stimulating factor

MCP-1 – monocyte chemoattractant protein 1

IFNγ – interferon gamma

TNFα – tumor necrosis factor alpha

IL - interleukin

Table 3. Secretion of Pro-inflammatory Cytokines by ASCs

So obesity results from a complex series of cellular events that ultimately increases the production of inflammatory adipokines within the tissue. These adipokines are known to further increase adipocyte hypertrophy producing a positive feedback system. This feedback system could be augmented further by the secretory activity of non-fat cells – i.e. the pre-adipocyte and even the ASC. Pre-adipocytes and adipocytes secrete many of the same pro-inflammatory factors listed above - with the exception of leptin and adiponectin, factors secreted by the adipocyte (reviewed in [235]). Furthermore, a review of the current literature

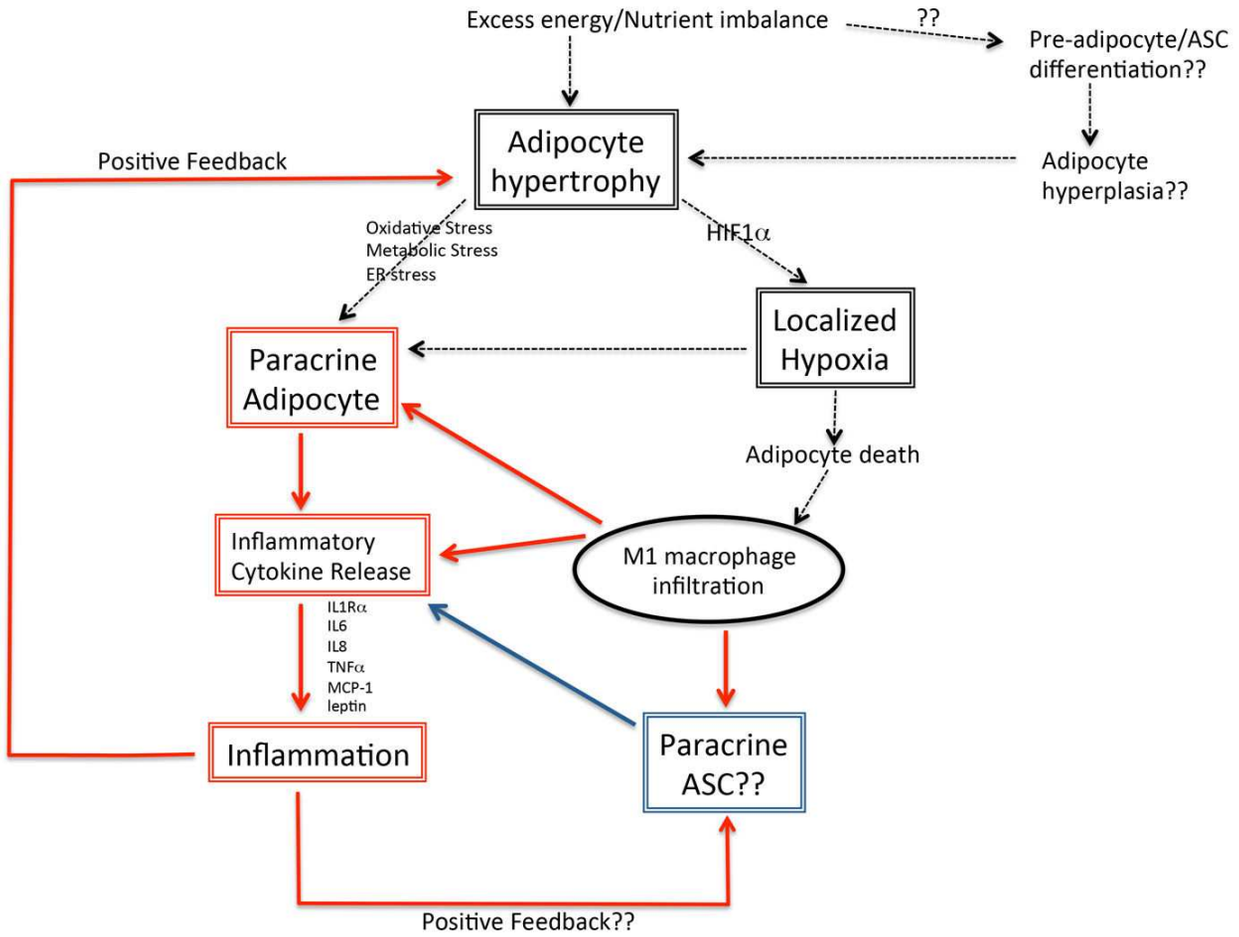

Figure 1. Possible interactions in obesity. Excess energy leads to development of hypertophic adipocytes. Hypertrophic adipocytes lead to the development of cellular stresses and hypoxia, via HIFI1α signaling, which can induce the adipocyte to release numerous pro-inflammatory cytokines. Hypoxia can also result in the death of adipocytes, inducing infiltration by pro-inflamatory/M1 macrophages into the adipose tissue. Paracrine activity by macrophages could affect the release of inflammatory cytokines from the adipocytes. In addition the macrophage may also release these cytokines directly. The resulting inflammation is likely to set up a feedback loop to enhance hypertrophic adipocyte development. The role of the ASC remains unknown in obesity but possible points of interaction could be the differentiation of ASCs, leading to adipocyte hyperplasia and the release of similar pro-inflammatory cytokines. Paracrine activity is shown as solid arrows.

turns up many studies that document the secretion of similar pro-inflammatory factors by ASCs (Table 3). It is possible that the secretion of inflammatory factors, like IL6 or TNFα, by ASCs may play a crucial role in inflammation and the development of obesity. Alternatively, it is possible that inflammation and obesity may result from "defective" ASCs that fail to secrete key anti-inflammatory factors such as IL-10 or have lost their ability to ameliorate oxidative stresses, thus allowing inflammation to go on unchecked. Unfortunately, the effect of inflammation and the ASC is under-represented in today's literature. Those studies that do exist document the inhibition of ASC adipogenesis under inflammatory conditions [248]. This is an interesting finding, as the ASC might be thought of as the logical source for adipocyte hyperplasia observed in obesity. However, if it is the paracrine activity of the ASC that plays a crucial role in the development or maintenance of obesity, then ASC differentiation capacity

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might be sacrificed in the name of maintaining this function. In light of what we know about adipocytes and pre-adipocytes in obesity, more in-depth studies on the ASC are certainly warranted.

A similar argument for ASCs could be made for other adipose disorders such lipedema and rare adipose disorders (RADs) such as Dercum's (aka Adiposa Dolorosa) [249] and Madelung's disease or Multiple Symmetric Lipomatosis (MSL) [250]. Lipedema (LD), or edema of the fat, is defined as the symmetrical accumulation of adipose tissue in the lower extremities [251]. Because the fat may also be painful as the disorder progresses, LD is often described in the same spectrum as Dercum's [252]. While lipedema and obesity share many similarities – leading to the misdiagnosis of lipedema in up to 15% of the population as obesity, there are some significant differences between LD and obesity. Specifically, excess fat accumulates almost exclusively in the lower limbs in LD and this adipose tissue is stubbornly resistant to loss through dieting [253]. LD is almost exclusively seen in women in their 30s or older, suggesting a hormonal component [251]. Despite these differences, the etiology of obesity and LD may share some commonalities, in that LD is thought to be mediated, in part, through hypoxia and the production of inflammatory cytokines (Figure 2). Like obesity, LD is initially characterized by adipocyte hypertrophy and hyperplasia [254], although the reason for this hypertrophy cannot be attributed to nutrient overload and currently remains unknown. This hypertrophy results in hypoxia, which is thought to result in inflammatory adipokine secretion and a putative positive feedback cascade as seen in obesity. Like obesity, LD fat is characterized by macrophage "crowns" in close association with hypertrophic and/or necrotic adipocytes [132]. These macrophages will almost certainly contribute to the inflammatory reactions occurring in LD fat. Furthermore, when examining adipose tissues isolated from Dercum's, similar immune infiltrations in association with perivascular cells and hypertrophic adipocytes are also seen, again, suggesting that LD and Dercum's may be points along the same spectrum [252]. In light of these commonalities with obesity, it would be logical to assume that the ASC would also play some critical role in mediating inflammation in LD or RADs through its production of paracrine factors. Unfortunately, these studies do not exist at this point.

Despite sharing many of the same characteristics, there are some important distinctions between obesity and LD that may also be at work. These distinctions are also likely to be found in RADs like Dercum's and Madelung's disease. Specifically, LD (and possibly Dercum's and Madelung's) is associated with defects in the microvasculature, together with lymphatic dysfunction [252]. Current theories propose that adipocyte hypertrophy leads to hypoxia, which results in increased angiogenesis. However, this angiogenesis is pathologic and the resulting capillaries are said to be "fragile" or "leaky" [255]. In support of this, perivascular cells, indicative of vascular damage, can be found in LD adipose tissue [254] and pathologic angiogenesis producing fragile capillaries have been found in many eye diseases [256], [257]. What produces this pathology is unknown but studies have shown that leptin can increase the number of fenestrations in capillaries [258] and increased plasma VEGF levels can be found in LD patients [259]. Increased plasma VEGF levels can also be found in LD patients [259], so it is possible that paracrine secretion from hypertrophic and hypoxic adipocytes could disrupt angiogenesis within LD adipose tissue. With studies showing ASCs capable of secreting

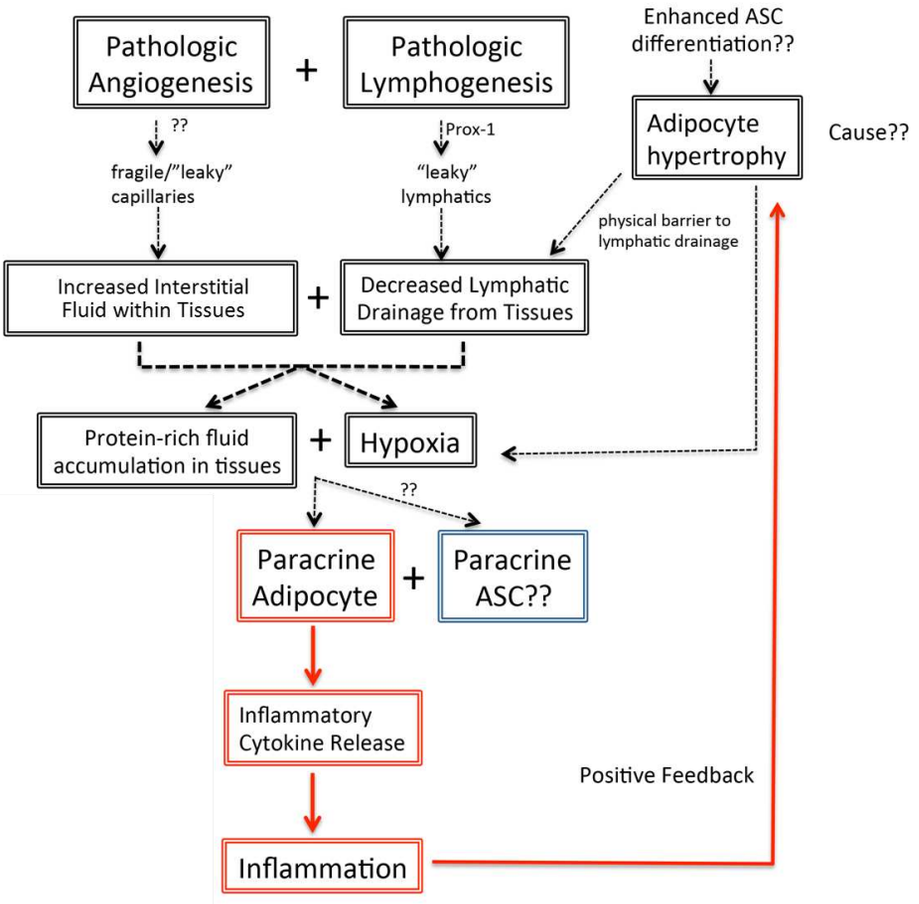

Figure 2. Lipedema. Development of lipedema may have numerous commonalities with obesity starting with the development of hypertrophic adipocytes. However, causation for this is unknown at this time may involve the ASC. As with obesity, adipocyte hypertrophy can lead to the development of hypoxia and the release of inflammatory cytokines from the adipocyte. Possible release of these factors from the ASC due to hypoxia is also shown. In addition, adipocyte hypertrophy is also accompanied by the development of "leaky" capillaries and lymphatics. While the cause of pathologic angiogenesis remains unclear, a role for the gene Prox-1 is though to be involved in lymphatic pathology. Increased filtration from capillaries, combined with poor lympmphatic drainage (due to hypertrophic adipocytes and the the leaking of lymph back from the lymphatic vessel) leads to an accumulation of protein rich fluids within the tissue. Fluid accumulation and hypoxia may induce pro-inflammatory cytokine release. Other mechanisms of obesity (e.g. macrophage infiltration) are also likely to be involved. Paracrine activity is shown as solid arrows.

numerous paracrine factors, including VEGF, and inducing endothelial differentiation and vessel formation, the question of whether the ASC plays a role in this vascular pathology should be asked. The fragile capillaries allow the filtration of protein-rich plasma into the interstitial space, driving the formation of edema [255]. In the early stages of LD, lymphatic drainage can keep up [260]. However with progression of the disorder, lymphatic drainage does decrease as the patient ages [253]. Added to this, the hypertrophic adipocytes are thought to physically restrict fluid drainage and the smaller lymphatic vessels themselves are thought to become "leaky", possibly through the appearance of microaneurysms in these vessels [253]. All of this results in the accumulation of lymph within the adipose tissue. Recent studies now suggest that "lymph can make you fat" [261]. In support of this, adipogenesis in vitro increases

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when cells are cultured in the presence of lymph [262], [263]. Furthermore, the removal of axillary lymphs nodes in individuals with breast cancer is frequently associated with increased fat deposition within the arm [263]. More recently, mice heterozygous for a mutation in the Prox1 gene not only exhibit leaky lymphatics, but develop obesity as they age [264]-[266]. What it is in the lymph that enhances adipogenesis is unclear. It simply could be the result of edema causing hypoxia, inflammation and adipocyte hypertrophy – not unlike obesity. Alternatively, factors in the lymph could directly induce the ASC to differentiate or the mature adipocyte to store more fat. Since lymph is interstitial fluid combined with emulsified fats, non-reabsorbed proteins and immunocompetent leukocytes, any of these factors could conceivably alter the behavior of the ASC. As it stands, more studies investigating the exact consequences of lymph accumulation on ASC and adipocyte behavior are needed.

So while the mechanisms may differ at points, at the basis of obesity, LD and RADs is inflammation. How the ASC participates in this inflammation remains to be seen, but the ASC could be used in the treatment of these disorders. If inflammation results in adipocyte hypertrophy, then ameliorating this process could decrease the size and number of these cells. In this regard, the anti-inflammatory, anti-oxidant properties of ASCs could be taken advantage of and enhanced in the hopes of mitigating the damaging effects of inflammation in these adipose disorders. However, before this could be attempted, more information is definitely required on the exact roles the ASC plays in adipose tissue formation and how these roles can go wrong when adipose disorders develop.

5. Conclusion

Since 2001, the number of studies characterizing and utilizing the ASC is truly staggering. It appears that the ASC is even passing the bone marrow MSC as the preferred adult stem cell for regenerative medicine. With its ease of isolation from adipose tissue, its availability within the tissue, its long term viability in culture and its persistence when implanted in vivo, the ASC is not only a great stem cell choice for studying mechanisms in vitro but for how it can regenerate tissues in vivo. In response, the studies using ASCs are incredibly diverse and range from their direct differentiation in regenerating tissues such as bone, muscle, nerve and liver to their indirect use in mediating inflammation, protecting nervous tissue and directing vascularization and wound healing through their production of paracrine factors. Finally, a truly exciting use for the ASC may be based on this paracrine activity, in that ASC appears to be easily engineered for the delivery of key factors capable of regenerating many tissue types and maintaining their health. Only time will tell how far the ASC will go.

Abbreviations

ASC = adipose-derived stem cell; EC = endothelial cell; LD = lipedema; MSC = mesenchymal stem cell; GFAP = glial fibrillary acidic protein; HLA = human leukocyte antigen; IR = ischemia

reperfusion; LVEF = left ventricular ejection fraction; MAP2 = microtubule associated protein-2; MLR = mixed lymphocyte reaction; PLA = processed lipoaspirate; RAD = rare adipose disorder; SVF = stromal vascular fraction; SC = Schwann cell; Tuj-1 = class III beta-tubulin; vWF = von Willebrand factor

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