Handbook of Regenerative Medicine and Tissue Engineering

Shay Fisher

Volume I

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Preface

This book has been an outcome of determined endeavour from a group of educationists in the field. The primary objective was to involve a broad spectrum of professionals from diverse cultural background involved in the field for developing new researches. The book not only targets students but also scholars pursuing higher research for further enhancement of the theoretical and practical applications of the subject.

The basic concept of regenerative medicine and tissue engineering is intriguing for physicians and scientists as it involves healing tissues or organ defects that the present medical practice finds difficult or impossible to cure. Tissue engineering involves cells, materials methods and engineering supported by appropriate physiochemical and biological factors to enhance or replace biologic functions. Regenerative medicine is a new division of medicine which aims to change the course of chronic disease and regenerate failing organ systems lost due to damage, age, disease and congenital defects. This book reflects state-of-the-art of these two disciplines at this time, as well as their therapeutic application. It discusses various topics related to stem cells in regenerative medicines. This book will prove to be a reference for physicians, scientists and students and an explanatory analysis for individuals in pharmaceuticals and biotech companies.

It was an honour to edit such a profound book and also a challenging task to compile and examine all the relevant data for accuracy and originality. I wish to acknowledge the efforts of the contributors for submitting such brilliant and diverse chapters in the field and for endlessly working for the completion of the book. Last, but not the least; I thank my family for being a constant source of support in all my research endeavours.

Editor

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Patricia Zuk

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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

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
Ii 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.