ADIPOBIOLOGY OF STEM CELL-BASED THERAPY: SECRETOME INSIGHT

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*Stem from the promise of stem cells therapeutic potential for a number of diseases, the regenerative medicine is reaching enthusiastic proportions nowadays. Current therapies include drug treatment, lifestyle modification, organ transplantation, RNA interference "breakthrough technology", and stem cell-based therapy. Research on stem cells is a multiplex challenge provoking both the attention and the confusion of biologists, biotechnologists, medical specialists, and politicians. By integrating various approaches of transcriptomics, proteomics and metabolomics, current adipobiology has identified more than 100 secretory proteins that are produced by the adipose tissue. These proteins designated adipokines include growth factors, cytokines, chemokines, neuropeptides and hypothalamic hormones/releasing factors. In addition, the adipose tissue's secretome contains steroid hormones, free fatty acids, prostaglandins, and endocannabinoinds. Moreover, adipose tissue is the source of adipose-derived stem cells (ADSC). Current interest in the ADSC stems from their multilineage differentiation potential, and ease of derivation in larger quantities using less invasive methods, compared with other stem cell types. The possible benefits of ADSC-based therapy may be mediated via cell proliferation/differentiation and/or paracrine mechanisms. The present review, focusing on adipose tissue secretory activity, also highlights the possible implication of ADSC in the therapy of various disorders, particularly neurodegenerative diseases, myocardial infarction and stroke, along with gut and liver diseases. **Biomed Rev** 2010; 21: 57-63.*

Key words: adipokines, adipose tissue, diseases, growth factors, secretion

INTRODUCTION

Crohn's disease: a prototype of adipose tissue-related disease

Dr Burrill B. Crohn (1884-1983) with his colleagues Leon Ginzburg and Gordon Oppenheimer published an article entitled "Regional ileitis: a new clinical entity" in 1932 (cited in 1), the disease being later coined after his name. Notably, these three authors described hypertrophy of mesenteric adipose tissue adjacent to the part of inflamed intestine. Insightfully, *Mycobacterium avium paratuberculosis* infection was etiologically linked to "Regional ileitis" by Crohn himself, now the story being revitalized (1). Arguably, the contribution of adipose tissue to the pathogenesis of infectious diseases is gaining attention. For instance, adipose tissue is one of the major hosts of *Trypanosoma cruzi* infection in Chagas disease (2) and of *Mycobacterium tuberculosis* (3).

The present article attempts to review current concepts of adipose-derived stem cells (ADSC), focusing on adipose tissue secretory activity, particularly its paracrine phenotype. We also highlight the possible implication of ADSC in the therapy of various disorders, particularly neurodegenerative diseases, myocardial infarction and stroke, along with gut and liver diseases.

ADIPOSE TISSUE

At structural and functional level, white and brown adipose tissue (WAT and BAT, respectively) are traditionally distinguished. Although the function and topology of BAT have been re-evaluated recently (4,5), adipose tissue ("WAT" will be assumed from hereon) is generally partitioned into two main large depots with visceral and subcutaneous location, and many small depots associated with various organs including heart, blood vessels, lymph nodes, eyes as well as parasellar region in the brain and epidural space in the spinal cord. Adipose tissue is a dynamic system, consisting of adipocytes and non-adipocyte cellular elements, including stromal, vascular, nerve and immune cells. These cells may input, process, and output information, a sophisticated biological module resembling brain, particularly hypothalamus (6-8).

At birth, the average-size infant has approximately five billion adipocytes, whereas their total number becomes approximately 80 billion in adults. Adding more than billion fibroblasts, mast cells, lymphocytes, dendritic cells and macrophages makes the whole body adipose tissue a major secretory organ of humans. Adipose tissue modulates whole body metabolism by controlling insulin sensitivity and circulating glucose and fatty acid levels in tissues such as muscle, liver and brain (8-15).

The most momentous challenge that has occurred in the field of adipose tissue research has been the discovery of leptin (from Greek *leptos*, meaning thin), an adipocyte-secreted *Ob* gene-encoded protein, in the end of 1994 (8). This became an acute trigger for the current development of adipobiology, particularly for the studies on cell protein secretion in adipose tissue (10,13-15). Hence the current paradigm framed by the two Nobel Prize winners George Palade and Gűnder Blobel regarding the rough endoplasmic reticulum-Golgi complex secretory pathway and the signal hypothesis, respectively, began to be explored in adipose tissue secretion, demonstrating that not only adipocytes but also other adipose cell types express a high secretory phenotype (9-16). For instance, 259 proteins were identified in the cultured human visceral adipose tissue 108 of them being secretory proteins (14), that is, bearing a N-terminal secretion signal peptide. Likewise, another in-depth proteome study on 3T3-L1 adipocytes identified 3 287 proteins (11), at present the largest proteome map of adipocytes.

According to current cell biology understanding, the process of protein secretion is mediated by synthesis, post-translational modification and folding (in the lumen of rough endoplasmic reticulum), sorting and targeting to final destinations such as plasma membrane, nucleus, intracellular organelles, and exocytosis. Briefly, the three major types of secretory proteins are plasmalemmal, intracellular (imported) and exported proteins (reviewed in 13,15,17), the adipokines being an example of the latter class of secretory proteins.

Further, non-rough endoplasmic reticulum-Golgi complex pathways using multivesicular endosome-derived exosomes and plasmalemma-shedding microparticles termed ectosomes are also appreciated in recent studies on adipo-secretion (15,16,18). Hence, both exosomes and ectosomes should also be considered the constituents of adipocyte secretome. Importantly, through endocrine and paracrine pathways these nano-sized structures translocate several membranes, cytosolic and nuclear proteins as well as adiponectin, exosomes being recently coined "adiposomes" (18).

In effect, systems adipobiology approach with integrated transcriptomics, proteomics and metabolomics identified more than 100 secretory (endocrine and paracrine) proteins designated adipokines (9-15). Trayhurn and Wood (10) conceptualized the proteome of adipose tissue as adipokinome,

whereas the whole spectrum of adipose secretory products was designated secretome, the latter embodying both proteins (adipokines) and non-proteins such as free fatty acids, steroid hormones (19), endocannabinoids (20) and nitric oxide (21) among others. Using current methodologies of adipoproteomisc (11,14,15), many newcomers are expected to arrive to adipose secretome.

Noteworthy, hypothalamic hormones/releasing factors, neuropeptides and neurotrophic factors are also synthesized and released from adipose tissue (6,7 and references therein) In effect, all components of adipose secretome may contribute to the regulation of numerous biological functions (appetite, satiety, energy homeostasis, lipid and glucose metabolism, inflammation, immunity, hemostasis, reproduction, aging, learning and cognition) as well as the pathogenesis of cardiometabolic (9,12,13,22,23), gastrointestinal (24-27), neurodegenerative (28-31) and other diseases (9,13) (Table 1).

In brief, the adipose tissue secretome is a potent producer of multiple pleiotropic factors; adipokines with a possible neuroregenerative potential are listed in Table 2. From the perspective of regenerative medicine, such a super-sized secretome may allow transplanted ADSC to exert a strong paracrine activity over the injured tissue. With the use of various bioengineering technologies, this activity may be directed in a tissue- and disease-specific manner.

ADIPOSE-DERIVED STEM CELLS

"In science, as in life, there seem to be three kinds of problems, which we classify as "easy", "difficult", or "impossible". "Easy" problems in science are those in which concept and methodology both appear to be adequate; "difficult" problems are those in which either concept and/or methodology appears to be deficient; "impossible" problems are impossible" –in 1964, the famous Oscar Hechter, together with Halkerston, wrote in their review "On the action of mammalian hormones" (32). In our present case dealing with the adipose secretome in ADSC-based therapy, the problem may be classified as a difficult, but not impossible problem.

Although the currently popular concept of stem cells can be traced back to the end of the 19th century (reviewed in 33), the potential of ADSC in regenerative medicine has only been appreciated recently (34-45).

Different stem cell populations have been intensively studied in the last decade as a potential source of new cells, for example, cardiomyocytes that can ameliorate the injured

Table 1. *Selected list of diseases and ADSC-based therapy*

Cardiometabolic diseases
Myocardial infarction
Stroke
Diabetes mellitus

Neurodegenerative diseases
Alzheimer's disease
Parkinson's disease
Huntington's disease
Multiple sclerosis
Peripheral nerve injuries

Gut and liver diseases
Crohn's diseases
Liver cirrhosis
Perianal fistula

Others
Rheumatoid arthritis
Erectile dysfunction
Soft tissue injuries
Chronic joint diseases
Duchenne muscular dystrophy

Table 2. *Selected list of "neuroregenerative" adipokines**

Nerve growth factor
Brain-derived neurotrophic factor
Ciliary neurotrophic factor
Glial cell line-derived neurotrophic factor
Vascular endothelial growth factor
Hepatocyte growth factor
Transforming growth factor-β
Granulocyte and macrophage colony stimulating factors
Angiopoietin-1
Insulin-like growth factor
Steroids
Leptin
Neuropeptide Y
Apelin
Metallothionein-I,-II,-III

* For references (6,7,66,68-70).

myocardium and eventually restore cardiac contractility, neural cells that can rescue injured brain and peripheral nerves.

Stem cells can generally be classified into embryonic and adult form; a variation of the former is so-called induced pluripotent stem (iPS) cells, whereas bone marrow-, umbilical cord- and placenta-derived stem cells as well as ADSC and skin progenitor cells are examples of adult stem cells. For a long time, embryonic stem cells were thought to be the only source of pluripotency, a dogma that has been challenged during the last decade. The derivation of human stem cells from pre-implantation embryos (specifically, from the inner cell mass of the human blastocyst at day 5 or 6 of the early embryo development) raised great expectations for their use in regenerative therapy (33). However, ethical concerns, teratocarcinomas formation upon transplantation and immunological risks became serious limitations at the clinical settings. A feasible alternative option might be provided by auto-transplantation of ADSC or skin-derived precursor cells as easily accessible and ethically acceptable source of stem cells.

The stromal vascular fraction of adipose tissue contains stem cells, T-lymphocytes, anti-inflammatory macrophages, endothelial precursor cells, and preadipocytes. In cell culture conditions, ADSC display an impressive developmental plasticity, including a multilineage differentiation potential; they can differentiate into bone, cartilage, muscle and neuronal cells (46-55). In addition, the ADSC are capable of expressing cholinergic molecules which could be a great news for reducing the burden of Alzheimer's disease (41). Noteworthy, a subset of adipocytes may originate from the neural crest cells (46,47).

Adipose-derived stem cells can be obtained by a less invasive method and in larger quantities compared with bone marrow-derived stem cells and neural stem cells. By liposuction, a common surgical operation, adipose tissue can be harvested in large quantities with minimal side effects from several regions of the body. On average, 100 ml of human adipose tissue yields about 1×10^6 stem cells, or about 5×10^5 stem cells could be obtained from 400 to 600 mg adipose tissue (35,36,43). Also, the flow cytometry analysis has previously reported that ADSC express high levels of stem cell-related antigens (CD13, CD29, CD44, CD105, and CD166) and stem cell-related transcription factors.

Understanding signaling pathways that drive proliferation, transdifferentiation and secretion of ADSC is of great importance for controlling their behavior. Applying efficient cell engineering protocols including encapsulation (for example, in microspheres) of various "stem cell growth factors" released into the medium of cultured ADSC may encourage their tissue-specific differentiation potential. Understanding various approaches involving both the transduction and the pharmacology of ADSC could further boost their utility. For instance, (*i*) stem cells transduced with tyrosine hydroxylase and other related genes could function as biological minipumps to enhance the dopaminergic neurotransmission after grafting (56), (*ii*) lovastatin, a cholesterol-lowering drug (57), and berberine, a plant antioxidant (58), both prevent ischemia-induced apoptosis in mesenchymal stem cells, and (*iii*) valproic acid (an anticonvulsant, mood-stabilizing and anticancer drug exerting histone deacetylase inhibitory action) affects neural stem cell proliferation (59) .

ADSC: AN OLD REMEDY?

Recent evidence shows that *Homo obesus* (60, cf. 61,62) is much prone to expressing a surprisingly high number of diseased phenotypes, including those discussed herein (24-31,63-65). Symbolically, if ADSC transplantation may indeed bring therapeutic effects in patients, this might be a science-based rescue of the obese humans suffering from various cardiometabolic and neurodegenerative diseases. Indeed, reminding us of *similia similibus curantur* (from Latin, meaning "like cures like"), a homeopathic axiom introduced by Dr Samuel Hahnemann (1755-1843) in the first edition of his book *The Organon of the Healing Art*.

In conclusion, considering the ease of derivation and ethical aspects, ADSC could indeed be a better source of stem cell (66) than bone marrow-derived stem cells (67). Translated into the topic of present review, this may sound as follows: adipose secretome is better secretome than bone marrow secretome. In brief, the regenerative potential of ADSC's secretome requires further investigation and appreciation.

Altogether, "the mechanisms we have proposed may be incorrect, but they can be tested; if found untenable, they can only be replaced by "better" mechanisms" – Hecther and Halkerston wrote in their 119 page-long chapter (32).

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