



The role of stem cells in anti-aging medicine

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Abstract Aging is the result of two overlapping processes, “intrinsic” and “extrinsic.” Intrinsic structural changes occur as a consequence of physiologic aging and are genetically determined; extrinsic relates to exposure to harmful events and habits, like smoking, bad diet, alcohol consumption, lack of sleep, stress, sun exposure, environmental pollution, etc. Aging may be decelerated by improving bad habits or treating signs of aging with various esthetic methods, food supplements, and antioxidants. It is believed that we cannot stop aging entirely due to the intrinsic part, which leads to irreversible cell damage, as well as tissue and organ damage due to their limited ability to regenerate. Stem cells and their ability to exhibit telomerase activity, to self-renew, and to differentiate into all three embryonic tissues challenges aging as a process, which is not inevitable and can even possibly be reversed. Stem cells can promote regeneration of aged tissues and organs by replacing apoptotic and necrotic cells with healthy ones. In addition, they can have antiinflammatory and antiapoptotic properties by paracrine-secreting growth factors and cytokines on the site of administration. Autologous adipose-derived stem cells are the most promising because they can be easily harvested in huge numbers with minimally invasive liposuction and, as such, represent a powerful tool in anti-aging and regenerative medicine. In this contribution, the author discusses their properties and application in clinical practice.

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Introduction

Advanced age in humans is considered to be the greatest risk for a range of diseases, including cancer, Alzheimer’s disease, cardiovascular diseases, atherosclerosis, and the metabolic syndrome, to mention few. There exists the possibility that targeted approaches to aging will delay the onset of many metabolic and biochemical properties that contribute to morbidity in the elderly and, therefore, to achieve longer life expectancy, or at least a healthier and more comfortable aging process.¹

Aging

Aging is the result of intrinsic and extrinsic overlapping processes. Intrinsic structural changes occur as a consequence of physiologic aging and are genetically determined.^{2,3} The rate of aging is significantly different among different individuals and even among different anatomic sites in a single individual. Many theories have tried to explain the aging process, but the most plausible of these concentrate on DNA damage and repair processes, which induce genome-wide epigenetic changes leading to cell senescence, loss of proper cell function, and genomic aberrations.⁴ The signals of DNA damage lead to the following three possible responses regarding cell fate: (1) transient cell cycle arrest (repair), (2) stable cell cycle arrest (senescence), or (3) cell death (apoptosis). Intrinsic (genetically determined or chronologic) and extrinsic (ultraviolet radiation and toxic exposure-

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mediated) aging processes overlap and are strongly related to increased generation of free radicals in cells, tissues, and organs. The underlying mechanism of both processes is increased oxidative stress, which is probably the single most harmful contributor to aging, leading to loss of cells and the extracellular matrix.⁵

Manifestations of aging

The clinical manifestations of intrinsic aging reflect the balance between severity of tissue and organ damage and their regenerative abilities. All proliferating and terminally differentiated cells are susceptible to harmful events that lead to intrinsic aging.⁶

The signs of aging generally start early, at the age of 40 years. Most organs, tissues, and cells gradually become aged and less efficient. The skin becomes thin, transparent, less elastic, more wrinkled, and loose. There is a loss of underlying fat of the face, leading to hollowed cheeks and eye sockets. Perspiration is insufficient. It is normal for hair to gradually thin on the scalp, in the pubic area, and in the armpits. As melanocytes decline in number, hair becomes gray. Nail plates gradually become thinner.⁷ By the age of 80 years, there are visible changes in posture due to compression of joints, spinal bones, and spinal disks. Regarding hearing, high-frequency sounds become harder to hear.

Most elderly patients develop presbyopia (the lenses in the eyes become less flexible) and need reading glasses. As patients age, they sleep less and not as deeply as when they were younger. The bones become less dense and strong and are prone to break more easily. There is also more body fat and less muscle mass due to hormonal changes and slowed metabolism. Memory commonly declines. An aged individual does not remember names well and has poor recent memory recall. The heart becomes less efficient and needs to overcome bigger resistance as it ages and consequently starts to fail. The lungs supply less oxygen over time. The kidneys decline in function, and waste products of metabolism build up and cause toxicity. Sexual dysfunction is common in men, and women undergo a number of menopausal changes linked to lower estrogen production. In addition, an inactive lifestyle and unhealthy diet increase the risk of chronic diseases, such as coronary artery disease, osteoarthritis, high blood pressure, obesity, osteoporosis, type 2 diabetes, and cancer.

Stem cells

Stem cells are capable of extensive self-renewal and expansion and have the potential to differentiate into any type of somatic tissue.⁸ They can be used in regenerative medicine, reconstructive surgery, and tissue bioengineering and can be derived from various tissues. Embryonic stem cells are derived from human embryos from couples who undergo

in vitro fertilization, raising concerns about the ethics and the possibility of rejection. In addition, there is a concern of rejection reactions in nonrelated donors.⁹ Induced pluripotent stem cells are derived from modified differentiated adult somatic cells and have similar properties as embryonic stem cells. They are more acceptable because they are not derived from human embryos but involve major genetic modifications in *in vitro* conditions before they can be used for research and in clinical practice.^{10,11}

Adipose-derived stem cells

Autologous adult stem cells are immunocompatible, and there exist no ethical concerns related to their use. Multipotent mesenchymal stem cells (MSCs), which have similar characteristics to bone marrow–derived MSC, are nonhematopoietic cells from mesoderm and are present in the following postnatal organs and connective tissues: trabecular bone,¹² periosteum,¹³ synovial membrane,¹⁴ skeletal muscle,¹⁵ skin,⁸ pericytes,⁹ peripheral blood,¹⁶ deciduous teeth,¹⁷ periodontal ligament,¹⁸ and the umbilical cord.^{19,20} Adult stem cells derived from those tissues would require *ex vivo* expansion or manipulation before they could be used clinically, because their number in all of those tissues is low.

Multipotent stem cells within adipose tissue, termed adipose-derived stem cells (ASCs),²¹ are one of the most promising stem cell populations identified thus far, because human adipose tissue is easily harvested by minimal liposuction and does not cause discomfort in the patient. The autologous ASCs have been shown to be safe and effective in preclinical and clinical studies.^{22,23} To date, a number of scientific contributions on ASC biology and their use in regenerative medicine have been published, and their efficacy has been determined in several clinical trials.

Localization and cellular characteristics of ASCs

Adipose tissue is composed mainly of adipocytes (fat cells), which are clustered into fat lobules.²⁴ Adipose tissue consists of mature adipocytes (>90% of the tissue volume), and a stromal vascular fraction (SVF), composed of preadipocytes, fibroblasts, vascular smooth muscle cells, endothelial cells, resident monocytes and macrophages, lymphocytes, and ASCs.^{25,26} Characteristics of ASCs differ according to the location of the harvested adipose tissue. Most resistant to apoptosis are ASCs harvested from superficial abdominal regions, followed by those harvested from medial thigh, trochanteric, and superficial deep abdominal depots.²⁷ The density of stem cells varies among different locations and types. They are most abundant in subcutaneous compartments of white adipose tissue compared with visceral fat.²⁸ ASCs have been found within the brown adipose tissue, which possess skeletal myogenic differentiation potential.²⁹ Freshly

isolated SVF is a heterogeneous cell population that includes ASCs, endothelial cells, vascular smooth muscle cells, pericytes, and hematopoietic cells in uncultured conditions.³⁰ Freshly isolated SVF and those after few divisions express higher levels of tyrosine-protein kinase Kit, human leukocyte antigen-DR, and stem cell-associated markers (eg, CD34), and lower levels of stromal cell markers.^{20,30-46} As they proliferate, they lose the CD34 surface antigen.⁴² ASCs, which express CD34⁺ have a greater proliferative capacity, whereas those that do not express CD34 are more plastic.^{31,47} They share many cell surface markers with pericytes and bone marrow MSCs.³¹ ASCs are most likely located within the perivascular region, because they express pericytes surface antigens.^{48,49} ASCs have the capability to divide, self-renew, and proliferate due to their telomerase activity, which is diminished as they age.⁵⁰ They do not exhibit immunosuppressive properties because they do not express HLA-DR antigens on their surface.⁵¹

The role of ASCs in regenerative medicine

Previous studies have suggested that ASCs exhibit their beneficial effects (angiogenesis, antiinflammation, and antiapoptosis) mostly by the secreted cytokines and growth factors rather than by their differentiation into various cell types.^{52,53} The ASC cytokines and growth factors have the potential to be used in cell-based treatments in regenerative medicine. A number of contributions have described the composition of the secretory factors of preadipocytes, ASCs, and adipose tissue.^{54,55} The cultured ASCs (after few divisions), secrete adiponectin, angiotensin, basic fibroblast growth factor, cathepsin D, CXCL12, granulocyte-macrophage colony-stimulating factor, hepatocyte growth factor, insulin-like growth factor-1, interleukins 6, 7, 8, and 11, pentraxin, pregnancy zone protein, retinol-binding protein, transforming growth factor beta, tumor necrosis factor alpha, and vascular endothelial growth factor.⁵⁴⁻⁵⁶

Proliferation capacity of ASCs

Previous reports have shown that ASCs double in number from 40 to 120 hours, depending on the age of the donor, type of adipose tissue (white versus brown), its location (subcutaneous versus visceral), the harvesting procedure, and culture conditions.^{21,41,57,58} Proliferation capacity of ASCs is highest in young individuals and is diminished with age. ASCs also gradually lose proliferative capacity with passaging in *in vitro* conditions.⁵⁸ Senescence of ASCs is similar to bone marrow-derived MSCs.⁵⁷ ASCs remain stable as they proliferate in culture and do not change their diploid karyotype.⁵⁹ Their proliferation can be stimulated by various growth factors, among which fibroblast growth factor 2 is the most important and is required for their self-renewal.⁶⁰⁻⁶² The proliferation of ASC can also be stimulated by platelet-

derived growth factor and oncostatin M.^{63,64} ASC proliferation can also be stimulated by growth factors supplemented by thrombin-activated platelet rich plasma,⁶⁵ human platelet lysate,⁶⁶ and human thrombin.⁶⁷

Differentiation potential of ASCs

ASCs have the capacity to differentiate into mesoderm, ectoderm, and endoderm lineage cells, although they are of mesodermal origin.^{68,69} They can differentiate into adipogenic,⁷⁰⁻⁷² osteogenic,⁷³ chondrogenic,⁷³⁻⁷⁵ myogenic,⁷⁶ cardiomyogenic,^{77,78} angiogenic,⁷⁹ tenogenic,⁸⁰ and periodontogenic lineages.⁸¹ There have been few studies published regarding their ectodermal differentiation potential. One group has described epithelial differentiation of cultured ASCs, which express the epithelial markers cytokeratin 8, cytokeratin 18, and E-cadherin.^{82,83} In another study, the differentiation of ASCs point toward a retinal pigmented epithelium ectodermal origin.⁸⁴ ASCs under cultured conditions can differentiate into neuronal or neuronal precursor cells.⁸⁵ Intravenous administration of ASCs in animal models on brain ischemia or hemorrhage demonstrated functional and histologic improvement.^{86,87} In addition, recent studies have revealed beneficial effect of intravenously administered ASCs in animals with a spinal cord injury because they migrated and partially differentiated into neurons and oligodendrocytes, and restored locomotor function.⁸⁸ Last but not least, it has been demonstrated that ASCs can differentiate into endoderm lineage cells. It has been published that ASCs have the potential to differentiate into hepatocytes,^{89,90} which raises the possibility for them to be used to reduce liver inflammation and treat liver fibrosis. In addition to hepatic differentiation, ASCs under *in vitro* conditions can differentiate in insulin, glucagon, and somatostatin producing cells.^{91,92}

Discussion

Apoptosis and cellular senescence (damaged cells that have lost the ability to divide) are considered important factors in aging and age-related diseases. Dead cells are replaced by new cells in the process of regeneration. They originate from stem cells, although their proliferating or differentiating capacity depends on the age of the donors; as people age, proliferation capacity of stem cells diminishes and leads to senescence. Rejuvenation and aging deceleration could be achieved by eliminating senescent cells, but their epigenetic reprogramming is not yet fully understood. Another approach to reset "the aging-clock" and to improve chronological aging is to replace stem cells through either intravenous or local administration.⁹³

Adult ASCs seem to be a powerful tool in regenerative medicine because they can differentiate into all three embryonic tissues. The number of isolated stem cells per gram of processed adipose tissue is significantly higher than in bone marrow,

which is another advantage. Routinely, 1×10^7 adipose stromal or stem cells have been isolated from 300 mL of lipoaspirate, with greater than 95% purity.⁹⁴ The average amount of ASCs in processed lipoaspirate is 2% of all nucleated cells.⁹⁵

ASCs can be harvested by minimal abdominal liposuction performed under local anesthesia and processed by medical devices, which ideally should be part of closed, sterile, and standardized systems; this is in an effort to avoid their toxicity, pyrogenicity, hemolysis, sensitization, and genotoxicity, and to maintain their viability.

ASCs are currently employed in reconstructive and esthetic medicine. They are used as a heterogenous cell suspension of SVF in breast augmentation and craniofacial soft tissue and bone restoration by their local application into the breast and the face. They can also be used in patients with severe heart failure by injecting them in coronary arteries, and in patients with musculoskeletal disorders by injecting them into affected joints, although those treatments are not standardized and widely accepted. In addition, there are many clinical trials on their usage in various conditions, such as peripheral vascular diseases, fistulas, diabetes mellitus, liver cirrhosis, lipodystrophy, spinal cord injury, fecal incontinence, degenerative arthritis, radiation ulcers, and graft-versus-host disease.⁶⁸ They may be successfully used in anti-aging medicine to decelerate and even revert aging.

Conclusions

ASCs seem to be a powerful tool in anti-aging and regenerative medicine, because they can differentiate into all three embryonic tissues and secrete many cytokines and growth factors. Currently, there are many clinical trials on their usage in various conditions, and the results are promising. They can be harvested by minimal liposuction in autologous donors and administered locally or intravenously on the same day without previous genetic manipulation or their expansion in *in vitro* conditions. The number of ASCs per gram of processed adipose tissue is much higher than in bone marrow, which is another advantage for their usage.

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