International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2020): 7.803

Potency of Secretome in Reproductive Regenerative Medicine

Megaratni Sandhi[1], Wimpie Pangkahila[2]

[1]Student of Doctoral Program, Faculty of Medicine Udayana University, Indonesia

[2] Post Graduate Program in Anti-Aging Medicine, Faculty of Medicine, Udayana University, Indonesia

Abstract: ***Aging will arise in all biological creatures. The process is including decreased function of various organs and physical alterations. According to anti-aging medicine, the process of aging considered a disease that can be prevented, treated, and even treated returned to its optimal state like still young. Reproductive aging is marked by total plasma testosterone levels in men decrease between 1-2% per year starting around age 40. The decreasing in testosterone is multifactorial. Adult stem cells especially MSC in the niche microenvironment of stem cells are the functional units of tissue homeostasis and regeneration. MSC media has a therapeutic effect obtained from the secretion and release of the trophic molecule, which is now known as the secretome. MSCs may support or attract components of the stem cell niche and / or mimic non-existent niche cell paracrine signals. IGF is present in the MSC secretome and promotes the production of testosterone by Leydig.***

Keywords: MSC, Secretome, Testosterone, Aging

1. Introduction

Aging will arise in all biological creatures. The process is including decreased function of various organs and physical alterations. According to anti-aging medicine the process of aging considered a disease that can be prevented, treated, and even treated returned to its optimal state like still young. Use of knowledge the latest medical knowledge and technology aim to extend live in good health [1].

Reproductive aging is marked by total plasma testosterone levels in men decrease between 1-2% per year starting around age 40. The decreasing in testosterone is multifactorial and age-related decrease in testicular testosterone can be due to a decrease in the number of Leydig cells and / or a reduction in steroidogenic abilities [2].

Adult stem cells exclusively Mesenchymal stem cells (MSCs) in the niche microenvironment of stem cells are the functional units of tissue homeostasis and regeneration. Niche is needed by stem cells in order to function because it plays a role in maintaining stem cell groups and regulating cell behavior according to the surrounding environment and long-distance signals [3]. The MSC media has a therapeutic effect obtained from the secretion and release of the trophic molecule, which is now known as the secretome. This review will cover the potency of secretomes which are produced by Stem Cell in Reproductive Regenerative Medicine.

2. Material

2.1 Reproductive Aging

Aging is a process that cannot avoided, where the decreased biological function depends on several factors, such as hormones, free radicals, glycosylation, genetic factors, decreased immune system, lifestyle and unhealthy diets, lack of habits good, environmental pollution, stress, and poverty [1].

According to anti-aging medicine, the aging process is considered as a disease that can be prevented, treated, and even returned to their optimal state like still young [1]. Anti-aging medicine is defined as part of medical science based on the use of science the latest medical knowledge and technology to conduct early detection, prevention, treatment, and improvement to the initial state of various dysfunctions, disorders, and diseases related to aging, which is intended for prolong life in a healthy state [1].

Utmost studies show that decreasing total plasma testosterone levels starting around age 40, although free testosterone decreases more rapidly (up to 3% per year) where SHBG levels rise simultaneously. The decrease in testosterone is multifactorial but can be divided into compensated primary hypogonadism (low / normal testosterone with high LH) primarily associated with aging and secondary hypogonadism (low testosterone with low LH) which does not appear to be age related but is associated with obesity. It also suggests that circulating androgens are in response to a decrease in LH that increases with age [2].

Age-related decrease in testicular testosterone can be due to a decrease of Leydig cells and or a reduction in steroidogenic abilities. Many studies in human populations report that the number of Leydig cells decreases with age. However, recently found no age-related changes in the number of Leydig cells. Counting the number of cells in the testes is prone to technical problems, this has influenced the results of previous studies so that the results are uncertain. Better evidence of degenerative changes in cells includes lipofuscin granules, cytoplasmic or intranuclear crystal inclusions, reduced smooth endoplasmic reticulum, and smaller and fewer mitochondria [2].

2.2 Stem Cell in Reproductive Organs

Researchers are looking for and trying to understand why some cells and organs in the human body are able to repair themselves and some are not. Currently that search is focused on the stem cell field. Stem cells are a special type of cell with the ability to rebuild themselves (self-renewal) and at the same time to form specialized cells. Stem cells are characterized by the ability to differentiate into a wide variety of specific cell types. Its ability to proliferate together with its ability to differentiate into certain types of cells is what is unique. There are two main groups of stem cells according to the source, namely embryonic stem cells isolated from the inner cell mass of the embryo, and adult stem cells (ADS) isolated from adult tissue [3].

Adult Leydig cells (ALC) are thought to originate from non-steroidogenic mesenchymal cell (nearby to the seminiferous tubule). Non-steroidogenic mesenchymal cell differentiate into testosterone-producing cells during neonatal period. Currently, ALC replace fetal Leydig cells, although fetal strains remain as a small population in the adult testis. During development of male sexual characteristics, ALC reproduces until puberty. During adulthood, Leydig cells are a steady population that is hardly changed by either division or apoptosis [4].

In 1982, Richardson et al. suspect the role of peritites as progenitors for adipocytes during tissue injury. Perisit has been shown to regenerate Leydig cells in drug-injected animal models resulting in the death of Leydig cells. Immunohistochemical examination, found positive Cyp450 marker cells disappeared 3 days after drug injection, and seen again about 14 days after injection. Perivascular cells proliferated 2 days after intervention. These cells express nestin and α-smooth muscle actin (α-SMA), which is an indication of their perivascular phenotype. The newly formed Leydig cells also express the NG2 pericit marker and platelet-derived growth factor (PDGF). A possible explenation is that the location of stem cells is in a perivascular giving rise to progenitor cells in the paravascular area. Progenitor cells then proliferate and differentiate to produce different offspring [5].

SLC has been shown to be in the testes of adult mice. Injection of EDS, an alkylating agent which is a selective toxin for ALC in mice, can eliminate ALC completely and subsequently decreases the hormone testosterone. ALC is repopulated, and testosterone levels return to normal after a period of recovery. This proves that old Leydig cells can be replaced with active Leydig cells in old animals with EDS injection treatment. Repopulated cells produce the hormone testosterone at high levels such as Leydig cells in young animals. Another study showed reduced serum testosterone in mice with functional ALC deficiency, can be reversed by transplantation of a Hoechst-dim testicular population from normal mice into the testicular interstitial [6].

Most researchers have been confident about the presence of SLC during the entire life cycle based on these findings, although it is not possible to identify all of them because of the lack of specific cell lineages or surface markers to date. In 2006, Hardy and colleagues purified SLC from the testes of neonatal mice by selecting the negative LH receptor (LHR) and platelet-derived growth factor receptor (PDGFR) from a population of positive cells [7]. These cells express GATA4, a leukemia receptor inhibitor factor, and c-kit. These protein are essential for development of Leydig cell, but they are negative for 3β-HSD and do not yield testosterone. SLC which is cultured in media containing insulin-like growth factor-1 (IGF-1), LH, and thyroid hormone, are thought to reproduce undifferentiated for a long time and differentiate into testosterone-producing cells. These cells returned 3β-HSD-positive cells in the testes of mice treated with EDS, and it was decided that this cell population represented SLC. In this study it was also reported that PDGFRa positive peritubular cells showed SLC-like characteristics. The cells not only expressed the SLC marker, but also several pluripotent markers (Nanog and Oct-3/4) [8].

2.3 Potency of MSC Secretomes

Adult stem cells are the functional units of tissue homeostasis and regeneration. Niche is needed by stem cells in order to function because it plays a role in maintaining stem cell groups and regulating cell behavior according to the surrounding environment and long-distance signals. Resident stem cells must multiply and differentiate into functional cells to be able to participate in tissue renewal or recovery of damaged tissue. The differentiation process may depend on signals from the components of the stem cell niche, so coordinated niche repair is a priority to ensure adequate tissue regeneration [9].

Gnecchi et al., For the first time in 2005 revealed that MSC media has a therapeutic effect obtained from the secretion and release of the trophic molecule, which is now known as the secretome. Cell tracing analysis revealed that when transplanted, MSCs were not always damaged / dead. This suggests evidence that the secretome is considered a major tool in MSC-mediated repair and regeneration [9]. Secretome of MSC is described as a combination of a complex protein cytokines, growth factors, exosomes and microvesicles with therapeutic effects [9].

MSCs may support or attract components of the stem cell niche and / or mimic non-existent niche cell paracrine signals. IGF is present in the MSC secretome and promotes the production of testosterone by Leydig [10-11]. This hypothesis is consistent with the continued concept of the regenerative potential of MSC, where its role as an effector has been replaced by a role as a regulator that provides temporary paracrine stimulation to target cells and triggers regenerative processes in the tissue after damage occurs [12].

2.4 Secretome of MSC as Cell-Free Therapy in Regenerative Medicine

Secretome sourced from MSC is defined as a series of bioactive factors derived from MSC (including dissolved nucleic acids, proteins, extracellular vesicles (EV) and lipids) which are secreted into the extracellular space [13-

14]. EV derived from MSC contains a lipid bilayer that is enriched with various proteins (tetraspanin, integrins, ligands for cell surface receptors) allowing for intercellular communication, adhesion and endocrine effects [14]. A large number of bioactive molecules derived from MSC include genetic material (microRNAs (miRNAs), RNA and DNA), growth factors, immunomodulatory, and signal transduction proteins, and enzymes enveloped by a bilayer membrane [15]. EVs originating from MSC include microvesicles, apoptotic bodies, and exosomes (Exos), and can be differentiated based on their size and origin in cells [15-16]. Apoptotic bodies represent the largest EVs (> 1000 nm) destroyed during apoptosis. Microvesicles derived from MSC are nano-size EVs (100-1000 nm) that develop from the plasma membrane [15]. Exos is an EV originating from MSC with the smallest size (30-200 nm) originating from the endosome membrane called multivesicular bodies (MVB). After fusion of MVB with the plasma membrane, MSC-derived Exos are released into the extracellular environment. Afterward they exert biological effects by modulating multiple cell signaling pathways in target cells [16].

MSC-derived conditioned medium (MSC-CM) has a comprehensive environmental condition of soluble factors derived from MSC and vesicular elements [15-16]. Exos and MSC-derived microvesicles have overlapping size ranges, therefore the method currently used to separate these two EV sub-populations has varying degrees of success, so that when separation cannot be fully ascertained, the two encapsulated products are sourced from MSC. Collectively designated as EV originating from MSC. EV derived from MSCs can be carried to remote locations via biological fluids endocrine, and can modulate the function of immune cells, EC, pericits and other tissue resident cells [14]. EV originating from MSC-EV can fuse with the plasma membrane and deliver its contents to the target cell cytosol directly or MSCs interacts with target cells by a different mechanism. MSC-EV binds to membrane-bound receptors and triggers intracellular signaling which allows internalization of their content in target cells [15-16].

Numerous effects were witnessed in animal studies after MSC-CM and MSC-EV administration. The MSC-sourced secretome is able to bypass many of the boundaries of MSC-based therapies, including potential activation of allogeneic immune responses and undesirable differentiation. Secretome derived from MSC is practical for clinical setting because it can be produced tremendously from commercially available cell lines thereby avoiding invasive cell collection procedures [17]. In contrast to MSCs which must be expanded in culture to achieve optimal cell counts for transplantation, secretomes sourced from MSCs are readily available for the treatment of myocardial infarction, acute conditions including fulminant hepatitis, and cerebral ischemia [18]. Considering the logistical and biological advantages when compared to MSC-based therapies, MSC secretome administration is increasingly being considered as a new cell-free treatment for degenerative and inflammatory diseases [19]. The current use of cell transplant-free methods such as the use of the MSC secretome provides more advantages compared to stem cell transplantation, namely:

1) The use of secretomes resolves cell survival in transplantation.
2) Secretome has less expression of cell surface protein, thus providing less immunogenicity when compared to cells [14]
3) The use of secretome as a ready-to-use product significantly reduces the amount of cells requisite for transplantation (7×10^6 cells / kg.
4) Greater production are possible through the use of controlled laboratory environments (bioreactors) thus providing an improved bioactive compounds [9]
5) Economically and practically for clinical applications because it evades invasive cell collection processes.
6) The secretion of MSCs can be modified to preferred cell-specific effect.
7) The time and cost of expanding and maintaining the cultured stem cells can be greatly reduced and existing secretome therapy can be readily available for treatment.
8) Secretion of MSCs can be assessed for potency, dosage and safety similar to conventional pharmaceutical drug.
9) The storage of secretome can be carried out safely and without dropping the potency of the product, and avoiding the use of toxic cryoprotectant agents [14].

Thus, the use of the secretome has advantages over cell transplantation itself (as a whole or its components alone) as few clinical trials have been conducted so far using this concept have revealed feasibility and safety, with no reported side effects. This shows the secretome can be stored and transported efficiently as ready-to-use biological products [9, 20].

MSCs may support or attract components of the stem cell niche and / or mimic non-existent niche cell paracrine signals. IGF is present in the MSC secretome and promotes the production of testosterone by Leydig [10-11]. This hypothesis is consistent with the continued concept of the regenerative potential of MSC, where its role as an effector has been replaced by a role as a regulator that provides temporary paracrine stimulation to target cells and triggers regenerative processes in the tissue after damage occurs [12].

3. Conclusion

MSCs may support or attract components of the stem cell niche and / or mimic non-existent niche cell paracrine signals. IGF is present in the MSC secretome and promotes the production of testosterone by Leydig

References

[1] Pangkahila, W. Anti-Aging Medicine: Memperlamat Penuaan, Meningkatkan Kualitas Hidup. 2007, Jakarta, Kompas.
[2] O'Shaughnessy, P.J. The Human Leydig Cell. In: S.J. Winters and I.T. Huhtaniemi (eds.), Male Hypogonadism, Contemporary Endocrinology, Springer International Publishing AG. 2017, p. 23-39.
[3] National Institute of Health. Stem Cell information: The national institute of health resource for stem cell research. http://stemcells.nih.giv/info/basic/basics1.asp, 2 Juli 2017.

[4] Davidoff, M.S., Middendorff, R., Müller, D., Holstein, A.F. The Neuroendocrine Leydig Cells and their Stem Cell Progenitors, the Pericytes. Springer-Verlag, Berlin, 2009, p. 55-87.
[5] Meirelles, L.D.S., Caplan, A.I., NardiA, N.B. In Search of the In Vivo Identity of Mesenchymal Stem Cells. Stem Cells, 2008, 26: 2287-2299.
[6] Gimble, J.M., Katz, A.J., Bunnell, B.A.. Adipose-derived stem cells for regenerative medicine. Circ. Res.; 2007, 100(9): 1249–1260.
[7] Chen, H., Ge, R.S., Zirkin, B.R., Leydig cells: From stem cells to aging. Molecular and Cellular Endocrinology, 2009, 306: 9-16.
[8] Landreh, L., Spinnler, K., Schubert, K., Hakkinen, M. R., Auriola. S., Poutanen, M., Soder, O., Svechnikov, K., Mayerhofer, A. Human testicular peritubular cells host putative stem Leydig cells with steroidogenic capacity. J. Clin. Endocrinol. Metab. 2014, 99: 1227-1235.
[9] Teixeira, F.G., Salgado, A.J. Mesenchymal stem cells secretome: current trends and future challenges. Neural. Regen. Res., 2020, 15(1): 75-77.
[10] Sagaradze, G., Basalova, N., Kirpatovsky, V., Ohobotov, D., Nimiritsky, P., Grigorieva, O., Popov, V., Kamalov, A., Tkachuk, V., Efimenko, A. A magic kick for regeneration: role of mesenchymal stromal cell secretome in spermatogonial stem cell niche recovery. Stem Cell Research & Therapy, 2019, 10: 342.
[11] Youssef, A., Aboalola, D., Han, V.K.M. The roles of insulin-like growth factors in mesenchymal stem cell niche. Stem Cells Int., 2017:1–12.
[12] Hoogduijn, M.J., Dor, F.J. Mesenchymal stem cells: are we ready for clinical application in transplantation and tissue regeneration? Front. Immunol., 2013, 4: 144.
[13] Hoogduijn, M.J., Lombardo, E. Mesenchymal Stromal Cells Anno 2019: Dawn of the Therapeutic Era? Concise Review. Stem Cells Transl. Med., 2019, 8(11): 1126.
[14] Vizoso, F.J., Eiro, N., Cid, S., Schneider, J., Perez-Fernandez, R. Mesenchymal stem cell secretome: toward cell-free therapeutic strategies in regenerative medicine. Int. J. Mol. Sci., 2017, 18: E1852.
[15] Maguire, G. Stem cell therapy without the cells. Commun. Integr. Biol., 2013, 6: e26631.
[16] Vishnubhatla, I., Corteling, R., Stevanato, L., Hicks, C., Sinden, J. The Development of Stem Cell-Derived Exosomes as a Cell-Free Regenerative Medicine. J. Circ. Biomark., 2014, 3: 2.
[17] Ferreira, J.R., Teixeira, G.Q., Santos, S.G., Barbosa, M.A., Almeida-Porada, G., Gonçalves, R.M. Mesenchymal Stromal Cell Secretome: Influencing Therapeutic Potential by Cellular Pre-conditioning. Front. Immunol., 2018, 9: 2837.
[18] Cunningham, C.J., Redondo-Castro, E., Allan, S.M. The therapeutic potential of the mesenchymal stem cell secretome in ischaemic stroke. J. Cereb. Blood Flow Metab., 2018, 38: 1276–1292.
[19] Harrell, C.R., Fellabaum C., Jovicic, N., Djonov V., Arsenijevic, N.,Volarevic, V. Molecular Mechanisms Responsible for Therapeutic Potential of Mesenchymal Stem Cell-Derived Secretome. Cells., 2019, 8, 467.
[20] Sagaradze, G., Basalova, N., Kirpatovsky, V., Ohobotov, D., Nimiritsky, P., Grigorieva, O., Popov, V., Kamalov, A., Tkachuk, V., Efimenko, A. A magic kick for regeneration: role of mesenchymal stromal cell secretome in spermatogonial stem cell niche recovery. Stem Cell Research & Therapy, 2019, 10: 342.

Author Profile

Megaratni Sandhi Student of Doctoral Program, Faculty of Medicine, Udayana University