SMJ- Sohag Medical Journal, Vol. 28 No3) 2024

Print ISSN1687-8353 Online ISSN2682-4159

Review Article

Methods of Isolating Stem Cells of Different Origins: An Essay

Hasnaa Ahmed Abo Elwafa[1], Eman Hassan Ali Salama[1], Heba Mohamed Saad Eldien[2], Nermeen Mohamed Mostafa Maher[3]

1-Department of Clinical and Chemical Pathology, Faculty of Medicine, Sohag University
2-Department of Histology and Cell Biology, Faculty of Medicine , Assiut University
3-Physician in Assuit regional blood transfusion....Assuit university

Abstract

Stem cells are generally unspecialized cells that divide, can be grown in the laboratory, and have unlimited reproductive capacity. Embryonic stem cells arise from the inner cell mass of the blastocyst, while adult stem cells do the same. Undifferentiated beings are divided into two categories based on their principles and practical qualities. First: in the initial stages of undifferentiated organisms arising from the internal atomic mass of the impact cell. Second: adult stem cells. Embryonic cells, derived from fetal fluid, are another type of stem cell. They are important cells that can be modified. It is found in many types of tissues in fat, bone, muscle, liver and veins. Many researchers have tried to test each type of stem cell and find out how they can be used to treat different diseases based on this classification. Umbilical cord hematopoietic stem cells have also been used successfully to treat fetal germ cell hematology. Furthermore, the human umbilical cord is a promising source of underdeveloped mesenchymal cells. Collection is painless, which makes it different from bone marrow stem cells. It has antitumor properties, promotes tissue repair, regulates immune responses and has the ability to rapidly self-renew. Pluripotent stem cells are another type of reprogrammed cells that can be used to regenerate human organs and tissues. The aim of this review article is to find out how the use of stem cells from different sources in medicine can be facilitated by their isolation.

Keywords: Stem Cells; Isolation; Stem Cells Banking

DOI: 10.21608/SMJ.2024.299242.1481 Received: June 25, 2024 Accepted: July 13, 2024

Published: September 01, 2024

Corresponding Author: **Nermeen Mohamed Mostafa** E.mail: ymfm.101@gmail.com

Citation: **Nermeen Mohamed Mostafa**. et al., Methods of Isolating Stem Cells of Different Origins: An Essay

SMJ,2024 Vol. 28 No (3) 2024: 102- 112

Introduction

1. Stem Cells: Definition and Types

The first cells in the human body are called stem cells. Stem cells are the basic components of tissues, organs and the entire organism, as they can multiply infinitely and differentiate into any type of cell. They repair and regenerate damaged tissue throughout life after injury or disease. Reinforced or hereditarily adapted to the patient's example, these telephones together guarantee ideal anchoring and rapid distribution.[1]

stem-cells are primordial cells that are believed to be the ancestors of more than 200 different cell types in the human body. They can separate and produce different cells that can turn out to be exceptionally special. The cells are assumed to be SC, assuming that they possess the two associated characteristic properties: self-repair and pluripotency. Self-healing is the ability to grow indefinitely across fundamental divisions. The ability of the cell to produce all types of cells in the body is called pluripotency. These properties can be demonstrated in vitro by describing SC progeny through the flow of explicit cellular inheritance properties. This process is called differentiation.[2]

1.1. Bone-marrow stem cells

The main source of SC is tissue called bone marrow, which is found in the bones. It produces hematopoietic stem-cells and mesenchymal stem-cells. On the one hand, hematopoietic stem cells are the source of all blood cells: red blood cells that transport oxygen, megakaryocytes that produce platelets and interact with blood vessels and clotting factors, as well as cells of the immune system that defend them against microbes.[2]

1.2. Umbilical cord blood stem cells

It took decades to characterize hematopoietic stem-cells. The clear evidence that bone marrow was the source of these stem-cells strengthened the investigation and led to the discovery of another source of hematopoietic stem-cells in cord blood. After birth, the umbilical cord and placenta are examined for umbilical cord blood. Hematopoietic stem-cells and progenitor cells are abundant. From an immunological point of view, it contains cells that are even more immature than those of the bone marrow. This reduces the need for compatibility between donor and recipient. Reduces the number of irresistible specialists and wasted moves.[2]

1.3. Intestinal stem cells

Basic digestive microorganisms Villi, intestinal mucosal folds and crypts, composed of different types of cells and containing intestinal stem-cells, form the human intestinal epithelium. Because they are easily accessible and provide sustained and extremely rapid turnover of intestinal surface cells over a period of approximately five days, they have been used as a model to study the biology of heart failure in adults.[2]

1.4. Neural stem cells

stem-cells and brain pathogens are responsible for neurogenesis in undeveloped and adult organisms. Brain stem-cells produce new neurons involved in learning and memory. This is the site of adult neurogenesis and not early neurogenesis. Glial cells such as astrocytes, oligodendrocytes, and oligodendrocyte progenitor cells can be produced by neuronal stem-cells in the subventricular zone. These glial cells are found in all parts of the central nervous system and produce new myelinated oligodendrocytes throughout life.[2]

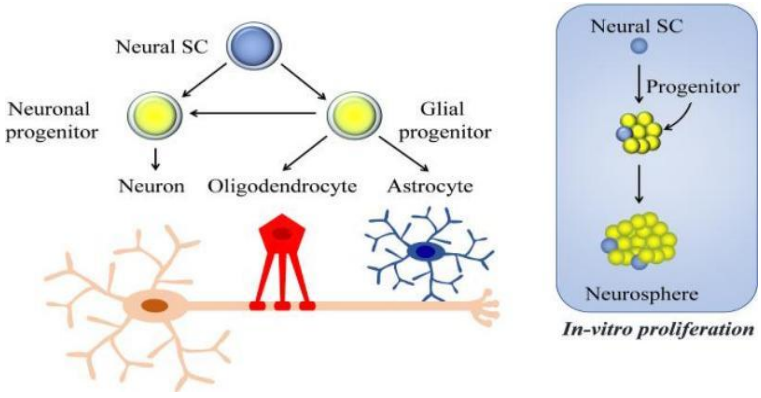

Figure 1 - Differentiation of neural stem-cells and neural progenitors in the adult human brain. Inset: In vitro proliferation is manifested by an aggregated cell structure, the neurosphere.[2]

1.5. Adipose tissue-derived stem cells

Adipose tissue, also called adipocytes, is a specialized tissue. There are two types of adipose tissue, whose adipocytes show morphological and useful contrasts: the more common of the two, consists of unilocular adipocytes, composed of a single lipid vacuole that increases the size of the adipose tissue. cytoplasm and destroys the nucleus. By the way, against the plasma film, hence the name unilocular adipose tissue.[3]

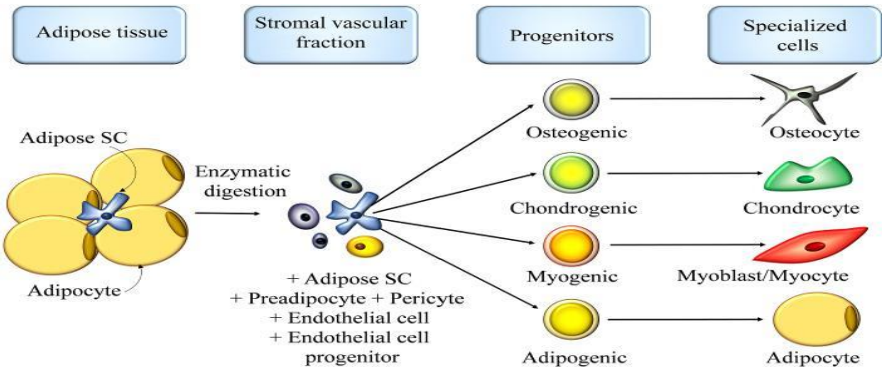

Figure 2 - Differentiation of adipose stem-cells and different lineages of progenitors. [5]

1.6. Induced pluripotent stem-cells or iPS cells:

By genetically reprogramming specialized somatic cells into cells with the capacity for self-renewal and pluripotency – two functional properties that make them very similar to human embryonic stem cells – we obtain laboratory-induced pluripotent stem cells also known as iPS cells. were recently discovered for the first time. [3]stem-cells in detail in the following sections, first examining adult stem-cells, then embryonic stem-cells, and finally induced pluripotent stem-cells to determine how they were discovered and what makes them unique. These stem-cells resemble a scientific platform that provides solutions to enormous expectations and medical needs. [4]

1.7. Skeletal muscle stem cells

Skeletal muscles are striated muscles that make up approximately 40% of human body weight. Skeletal muscles are made up of multinucleated contractile muscle cells called myofibers. Myoblasts, myogenic precursors of the mesoderm, fuse to form these myofibers. Most users are constantly present and connected to satellite cells, a group of SC groups. Based on interactions between satellite cells and the microenvironment of the cellular niche, skeletal muscle undergoes degeneration and regeneration after injury.[5]

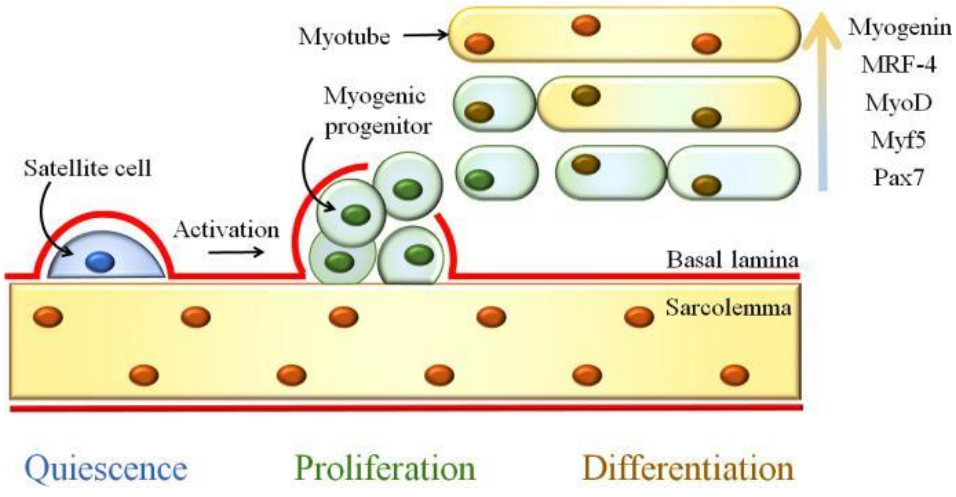

Figure 3 – Differentiation of muscular stem-cells from the satellite cell that leaves its quiescence and producesmyoblasts that differentiate into polynucleate and fused muscle fibers.[4]

1.8. Cardiac muscle stem cells

The heart is a postmitotic organ. However, as there is clear information about CS, it is clear that the pathogens are found in the musculoskeletal system of adults. The myocardium or muscular tissue of the heart, which includes the atria, ventricles, epicardium, and pericardium, houses a diverse collection of cardiac stem cells. These precursor cells do not contribute to the renewal of heart muscle cells. These are the specific muscle cells of the heart that are able to contract and maintain normal condition.[6]

1.9. Skin stem cells

It is the main concern of man and his protection against external hostilities, without pollutants and without the risk of hydration and proportionality of regulation and discrimination. Tissue composition: epidermis, epithelium defined externally and without vascularization, located above the dermis, connective tissue composed mainly of thick mixtures of fibers released by fibroblasts. A site of differentiation underlies the development of the basal body of the body and the outer layer of the epidermis by **Shim et al**.,[1] and **Orkin et al**.[7]

1.10. Embryonic stem cells

The discovery of underdeveloped stem-cells has significantly disrupted science in recent years. In 1981, Martin Evans, Matthew Kaufman, and Gail Martin isolated and cultured stem-cells from mouse embryos,[8] In 1998, James Alexander Thomson et al. discovered the same cells in humans.[9]

2. Review on Hematopoietic Stem Cells

The creators of definitive hematopoiesis, also known as the continuous production of blood cells throughout the life of an organism, are hematopoietic stem cells Hstem-cells. Each HSC can be tailored for basal part preparation according to design specifications: spheres are designed to enable oxygen transport, megakaryocytes and descending plaques through treatment of coagulation-solvent, because it is absorbed and adopts the cellular structure of the refractory macro, which manifests itself against microbial attacks[10]

3. Review on Stem Cell Banking Approaches

3.1. Cord Blood

In addition, they are suitable for use with 60 cm^3 of CB mayonnaise and for use with small e.g. children's blood volumes approximately 250 cm^3. Since blood is collected in a closed system, bag collection is preferred for most regulatory requirements. As an attack is unacceptable, bags should not be left unattended to avoid accidental contamination or uninterrupted blood circulation..[11] Proceed with caution and wait 5 minutes before removing the placenta after closing and separating the umbilical cord after reaching the umbilical cord.[12] The best way to use the placenta is to expel it very gently. [13]

On the **first** day of life 40 weeks, the umbilical cord should be used for 70-80 hours with a period between 850 and 1,100,106 nucleated cells personal experience. Most CB banks currently have many CB units in stock. Normally, you have to use your body to adapt to changing spaces. In commercial freezer bags, all but two separate compartments with volumes equal to 20% or 80% of the processed unit are now standard. Alternatively, if desired, small 2.5 cc cryogenic vials can be used to store a small amount of cells and plasma. [13]

3.2. Cord Tissue

Umbilical cord CT tissue, a direct source of Mstem-cells, is an additional source of stem cells that can be obtained simultaneously at birth. CT scans can be collected and archived as a future source of key microorganisms for regenerative medicine and tissue engineering. Despite Mstem-cells, CT also contains anterior endothelial and epithelial cells that could be useful for these application. [14,15]

Finally, a final rinse in PBS with 20% autologous plasma is performed, followed by resuspension in PBS/20% plasma for research, culture, or clinical purposes. Thus the described non-enzymatic digestion method can be used to isolate Mstem-cells from umbilical cord tissue. Currently, linear tissue pieces are washed thoroughly with PBS containing penicillin and streptomycin in a 100 mm Petri dish. To keep the minced tissue in its best shape, a 25 cm2 culture bottle is used. The fragments are collected after four to six days and cultured in a new flask. Cell colonies can be observed within 10-14 days. Cells from both flasks are then pooled and collected with trypsin-EDTA. Alpha-MEM expansion medium is then used to grow 25,000 cells in each additional 25 cm2 culture flask[16]

4. General Review on other Stem Cell Types

4.1. Mesenchymal Stem Cells Mstem-cells

The terms mesenchymal founder microorganisms Mstem-cells and bone marrow stromal cells are used interchangeably and refer to a population of plastic fibroblastic successor cells released by Percoll thickness centrifugation. In vitro and in vivo, Mstem-cells can develop into mesodermal cell lines such as adipocytes, chondroblasts, fibroblasts, osteoblasts, and skeletal myoblasts. Human Mstem-cells hMstem-cells cannot undergo more than 18 population doublings PD because they lack telomerase activity.[17]

4.2. Endothelial Progenitor Cells EPCs

The origin of endothelial cells, first described by **Asahara et al.** [18] are young endothelial cells circulating in the marginal blood PB. EPCs are essential components of the vascular system and transform into endothelial cells. In their groundbreaking study, translocated EPCs separated from human umbilical cord UCB blood were detected in the endothelium of newly formed blood vessels in ischemic areas. This demonstrates that a specific cell population in human blood is involved in the development of new blood vessels after ischemia..

Discussion

Tissues, organs and the entire organism are based on stem cells. They are the first cells in the human body and can reproduce indefinitely and differentiate into any cell type. During their lifetime, they repair and regenerate tissues damaged by injury or disease. [19]

According to **Ying et al**.[20], stem-cells are the first cells to form more than 200 different cell types in the human body. They are able to divide, resulting in the production of additional cells that can become highly specialized. If a cell is charging and pluripotent, it is considered SC. Self-renewal is the ability to reproduce indefinitely by simple division. Pluripotency means that the cell is ready to produce all types of cells in the animal.

According to **Iwasaki and Akashi.** [21], cell division determines whether stem-cells are charged or separated. stem-cells become generative cells or produce undifferentiated stem-cells. This process is determined by symmetric and asymmetric distributions. It is influenced by extrinsic and cell-inherent variables, such as improvements in the microenvironment.

Chambers et al.[22] concluded that IGF1 insulin-like growth factor 1 is a growth factor that can prevent differentiation. However, because they were not in the catalogs, he had to make the growth factors himself. They found that after several weeks the cells remained undifferentiated and contained hardly any nurse cells.

Friedenstein et al.[23] showed the way that Mesenchymal stem-cells can separate into bone and ligament, with the potential for use in the treatment of long bone cracks as one of their clinical applications. In addition, they share a number of characteristics, including the capacity for them to adhere to the plastic support.

In contrast to the study conducted by **Cheng et al.**[24], the digestive disorder persists throughout life. They are multipotent and produce enterocytes, goblet cells, endocrine cells and Paneth cells, all of which are important in the digestive lineage.

According to **Arrighi 4**, claims that the location where neural stem-cells generate new neurons involved in memory and learning is the locus of adult neurogenesis. In the subventricular zone, neural stem-cells can produce glial cells, which are also known as oligodendrocyte progenitors, astrocytes, and oligodendrocytes. These glial cells can be tracked down in all pieces of the focal sensory system and keep on creating new myelinated oligodendrocytes over the course of life.

Myofibers, contractile multinucleate muscle cells, are what make up skeletal muscle, according to **Yin et al.**[25] Each myofiber wires with satellite cells, a populace of strong stem-cells that can separate into various heredities of mesenchymal stem-cells, in spite of the way that the quantity of myofibers stays consistent. They differentiate into myocytes, adipocytes, and osteocytes when refined on a support of the kind used for Matrigel and resemble mesenchymal stem-cells obtained from bone marrow.

As demonstrated that the skin contains only a small number of stem-cells, including epidermal stem-cells, dermal mesenchymal stem-cells, hair follicle stem-cells, endothelial and hematopoietic stem-cells[1] As demonstrated that the string tissue itself, which is a prepared wellspring of Mstem-cells, is an additional wellspring of undeveloped cells that can be simultaneously obtained upon entering stem cells can be collected and stored as a potential source of stem cells for regenerative medicine and tissue engineering in the future.[14]

According to **Iwasaki and Akashi**[21], can produce both myeloid and lymphoid blood cell

lines and are highly capable of multidirectional differentiation..[26] to purify peripheral blood precursor cells or the bone marrow prior to transplantation. Using fluorescently labeled monoclonal antibodies that bind to specific cell surface proteins was one effective method for identifying hematopoietic stem and begetter cell HSPC cells. The subsequent methodology, which was being utilized all the while, was to choose BM cells by utilizing supravital colors that were nontoxic to cells. This was done in light of the differential restricting of the antibodies to the outer layer of the cell — high, low, and negative. Using a relative method for managing immunophenotypic strategies, cells were isolated considering a comparative high, low, and negative staining measures and a while later basically portrayed..

Conclusion:

Our audit article underlines enormous varability of various undifferentiated cells segregation strategies. A stem cell bank can store neural stem cells, hematopoietic stem cells, cardiac stem cells, mesenchymal stem cells, and epithelial stem cells for later use in the creation of specialized cell lines or cultures. Further in vivo and in vitro examinations are expected to improve cell recovery and conceivably uncover the best undifferentiated organism beginning for future medicines .

References

1-Shim, J., Lee, T. Shin, D.. Novel in vitro culture condition improves the stemness of human dermal stem/progenitor cells. Molecules and cells, 2013 , 366, 556-563.

2-Niwa, H., Ogawa, K., Shimosato, D., & Adachi, K.. A parallel circuit of LIF signalling pathways maintains pluripotency of mouse ES cells. Nature, 2009 , 4607251, 118.

3-Zhang, J., & Jiao, J.. Molecular biomarkers for embryonic and adult neural stem cell and neurogenesis. BioMed research international, 2015; 2015:727542. doi: 10.1155/2015/727542.

4-Arrighi, N.. Stem Cells: Therapeutic Innovations Under Control.Elsevier. Book, p 2018 , 1-178.

5-Wankhade, U. D., Shen, M., Kolhe, R., & Fulzele, S. Advances in adipose-derived stem cells isolation, characterization, and application in regenerative tissue engineering. Stem cells international, 2016; 2016:3206807. doi: 10.1155/2016/3206807.

6-Le, T. Y. L., & Chong, J. J. H.. Cardiac progenitor cells for heart repair. Cell death discovery, 2016 , 2, 16052.

7-Orkin, S. H., & Hochedlinger, K.. Chromatin connections to pluripotency and cellular reprogramming. cell, 2011 , 1456, 835-850

8-Evans, M. J., & Kaufman, M. H.. Establishment in culture of pluripotential cells from mouse embryos. Nature, 1981 , 2925819, 154

9-Thomson, J. A., Itskovitz-Eldor, J., Shapiro, S. S., Waknitz, M. A., Swiergiel, J. J., Marshall, V. S., & Jones, J. M.. Embryonic stem cell lines derived from human blastocysts. science, 1998 , 2825391, 1145-1147.

10-Riddell J, Gazit R, Garrison BS, et al. . Reprogramming committed murine blood cells to induced hematopoietic stem cells with defined factors. Cell; 2014 , 157: 549–564

11-Ghiaur, G., Ferkowicz, M. J., Milsom, M. D., Bailey, J.,Witte, D.,Cancelas, J. A., Yoder, M. C. and Williams, D. A.. Rac1 is essential for intraembryonic hematopoiesis and for the initial seeding of fetal liver with definitive hematopoietic progenitor cells. Blood 2008 , 111, 3313-3321

12-Miller PH, Knapp DJ, Eaves CJ.. Heterogeneity in hematopoietic stem cell populations: implications for transplantation. Curr Opin Hematol.; 2013 , 204:257-264

13-Chow, R.; Nademanee, A.; Rosenthal, J.; Karanes, C.; Jaing, T.-H.; Graham, M.L.; Tsukahara, E.; Wang, B.; Gjertson, D.; Tan, P.; et al. Analysis of hematopoietic cell transplants using plasma-depleted cord blood products that are not red blood cell reduced. Biol. Blood Marrow Transplant. 2007, 13, 1346–1357

14-Lindenmair, A.; Hatlapatka, T.; Kollwig, G.; Hennerbichler, S.; Gabriel, C.; Wolbank, S.; Redl, H.; Kasper, C. Mesenchymal stem or stromal cells from amnion and umbilical cord tissue and their potential for clinical applications. Cells 2012, 1, 1061–1088.

15-Zhang, X.; Hirai, M.; Cantero, S.; Ciubotariu, R.; Dobrila, L.; Hirsh, A.; Igura, K.; Satoh, H.; Yokomi, I.; Nishimura, T.; et al. Isolation and characterization of mesenchymal stem cells from human umbilical cord blood: Reevaluation of critical factors for successful isolation and high ability to proliferate and differentiate to chondrocytes as compared to mesenchymal stem

cells from bone marrow and adipose tissue. J. Cell. Biochem. 2011, 112, 1206–1218

16-Girdlestone, J.; Limbani, V.A.; Cutler, A.J.; Navarrete, C.V. Efficient expansion of mesenchymal stromal cells from umbilical cord under low serum conditions. Cytotherapy 2009, 11, 738–748

17-Muñoz-Elías, G., Woodbury, D., & Black, I. B.. Marrow stromal cells, mitosis, and neuronal differentiation: stem cell and precursor functions. Stem cells, 2003 , 214, 437-448.

18-Asahara, T., Murohara, T., Sullivan, A., Silver, M., van der Zee, R., Li, T., ... & Isner, J. M.. Isolation of putative progenitor endothelial cells for angiogenesis. Science, 1997 , 2755302, 964-966.

19-Frid MG, Kale VA, Stenmark KR. Mature vascular endothelium can giverise to smooth muscle cells via endothelial-mesenchymal transdifferentiation: in vitro analysis. Circ Res. 2002;9011:1189–96

20-Ying, Q. L., Wray, J., Nichols, J., Batlle-Morera, L., Doble, B., Woodgett, J., ... & Smith, A.. The ground state of embryonic stem cell self-renewal. Nature, 2008 , 4537194, 519

21-Iwasaki, H., & Akashi, K.. Myeloid lineage commitment from the hematopoietic stem cell. Immunity, 2007 , 266, 726-740

22-Chambers, I., Colby, D., Robertson, M., Nichols, J., Lee, S., Tweedie, S., & Smith, A.. Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. Cell, 2003 , 1135, 643-655

23-Friedenstein, A. J., Gorskaja, J. F., & Kulagina, N.. Fibroblast precursors in normal and irradiated mouse hematopoietic organs. Experimental hematology, 1976 , 45, 267-274 .of Sciences, 10840, 16825-16830

24-Cheng, H., & Leblond, C. P.. Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine I. Columnar cell. American Journal of Anatomy, 1974 , 1414, 461-479

25-Yin, H., Price, F., & Rudnicki, M. A.. Satellite cells and the muscle stem cell niche. Physiological reviews, 2013 , 931, 23-67.

26-Holyoake, T. L., Alcorn, M. J., & Franklin, I. M.. The CD34 antigen: potential clinical advantages of CD34 selection. Clinical Oncology, 1996 , 84, 214-221