

## Therapeutic Effects of Aging Stem Cells

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**ABSTRACT**

*Have researchers stumbled upon a way to create our own fountain of youth? Stem cells are unspecialized cells in the body capable of self-renewal and differentiation, ultimately responsible for the regenerative capacities of our body's tissues. Known changes that occur on the molecular and cellular levels of aging stem cells are: accumulation of toxic metabolites, DNA damage, epigenetic alterations, aggregation of damaged protein, mitochondrial dysfunction, changes within the stem cell niche and changes with the body's regulatory systems as a whole. Varying treatments, including the use of the antioxidant N-acetyl-L-cysteine, addition of mTERC to the cell, rapamycin treatment disengaging mTOR and the use of short-term and long-term calorie restricted diets, have shown promising effects in decreasing or reversing the level of damage caused to stem cells during the aging process. More research on how each of these mechanisms effects the cell, as well as determining if treatment is blocking the mechanisms causing these damaging effects or if treatment is simply treating the symptoms of these damages, is needed in order to further these treatments. The idea of an everlasting young pool of stem cells does not seem completely out of reach when the cellular and molecular changes occurring within the stem cell are studied.*

**Keywords**

Stem cells, Therapy, Aging.

**Introduction**

The world of biology is ever increasing with respect to increasing knowledge of stem cells. Stem cells are able to proliferate, grow, and then differentiate into functional tissues and organs. They are described as “clonogenic, self-renewing progenitor cells that have the ability to divide for an indefinite period and can give rise to one or more differentiated cell types” [1]. These cells differ from other cells in the body, often referred to as somatic cells, due to their ability to, first, self-renew. Self-renewal leads to differentiating and proliferating into a certain cell line, say for a certain tissue or organ type, which will then maintain the same degree of potency as the beginning stem cell [2]. Potency can be described as the cell's level of differentiation, also known as how specialized the cell is. This property of self-renewal is important for our body as whole because it shows stem cells are able to regenerate themselves, an important detail showing they can perform their second function throughout all of our life. The second main function of stem cells is to differentiate and give rise to cells with more specialized functions than their self. During this process, the potency of the

daughter stem cells decreases from the mother as the progeny begins to become more specialized as differentiation is increasing [2]. Together, these two functions of stem cells come together to create cells with powerful regeneration capacities; the ability to generate cells identical to themselves and the ability to generate cells more specialized than themselves.

Stem cells are often placed into three categorical groups by origin. The group that first occurring in human bodies during development are called embryonic stem cells (ESCs). ESCs can be found in the body throughout all stages of gestation [2]. The second naturally occurring stem cells, umbilical stem cells (USCs), can be found in cord blood and the final naturally occurring stem cells, adult stem cells (ASCs), often referred to as mature stem cells, are found in all humans after birth.

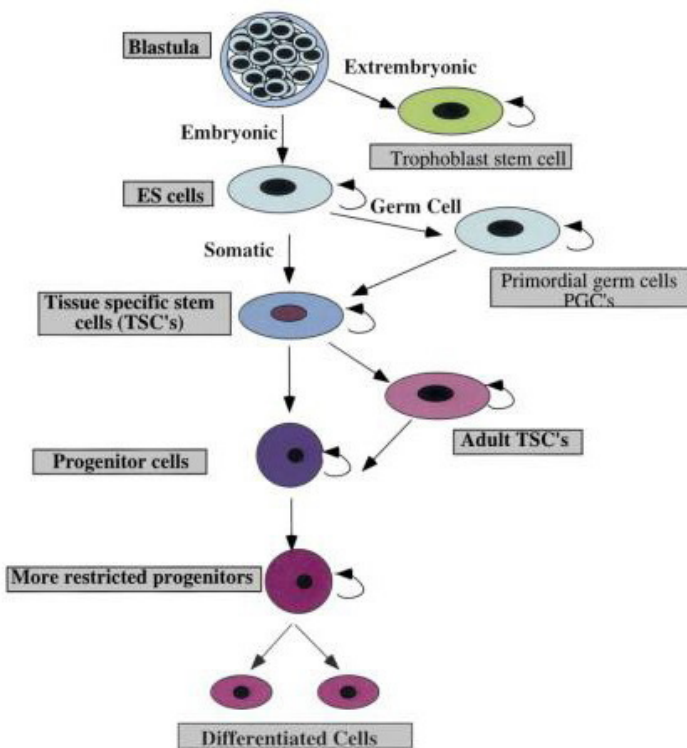
Stem cells can be placed into categorical groups based on their degree of potency. As mentioned earlier, potency is related to the stem cell's level of specialization, or the number of various cell types this single mother cell could potentially give rise to. The zygote, or fertilized ovum, retains the highest degree of potency as it can differentiate into all cell types and is therefore called

totipotent. Totipotent cells are capable of both giving rise to any cell type in the fetus or in the placenta. Potency starts to decrease in cells after the zygote, leading to some cells being labeled as pluripotent. Pluripotent cells, also known as the inner cell mass during development, can form any one of the three germ layers in the embryo, but cannot differentiate into placental cells. These pluripotent cells cannot give rise to their own organism, but simply only form a part of an already created organism [2]. Both of these cell types are believed, at this point, to be immortal, meaning that if placed in proper environments promoting strong activity of telomerase activity and DNA repair mechanisms, these cells could stay proliferative for an indefinite time period. The final class of stem cells is called multipotent stem cells. As a step down from pluripotency, multipotent stem cells have lost a large part of their capacity to self-renew and now only have a limited number of differentiations left [2]. Examples of multipotent stem cells that are well known are hematopoietic and embryonic mesenchymal stem cells.

to astrocytes or neurons in the hippocampus. Recent discoveries are now even showing stem cells found in the skin, myocardium, intestinal epithelium, mesoderm and much more. Studies using bromodeoxyuridine and other DNA precursors have enabled the findings that stem cells show a high degree of heterogeneity when production, differential and survival are compared [3]. This large degree of difference may be attributed to the stem cell adapting to meet the demands of the developing tissue to which it has formed a part.

Delving into the biochemistry of these stem cells, stem cells require several internal and external factors to promote their highly specialized functions. Stem cells have high telomerase activity, various cytokines, various growth factors and various external environmental factors that are required to maintain their self-renewal and differentiation properties [2]. These internal and external factors and changes occurring within the stem cell, along with their possibilities for future therapeutic opportunities will be the further topic of discussion in this paper.

### Stem Cells in Development



**Figure 1:** An outline of stem cell differentiation as it pertains to progenitors throughout the span of development. Tissue specific (medium-blue) stem cells and embryonic (light-blue) stem cells are highly examined throughout this paper. Figure taken from “Stem cells and aging: expanding the possibilities” [3].

An important reminder is that stem cells are formed during development, but are essential to humans throughout our life as they play a role in homeostasis of our organism [3]. Examples of these stem cells are hematopoietic stem cells that provide a continuous supply of blood cells or neural stem cells that give rise

### Discussion

Just as the number of stem cells is adapted to meet the demands of the developing tissues, so is the level of differentiation in each tissue also adapted. Figure 1 describes the fate of stem cells within the body, but stem cells may also stay quiescent, meaning they do not enter the cell cycle for a period of time, some biologists often call this phase G0 [3]. Having a pool of cells in this G0 period has shown to be crucial when the body becomes stressed and extra reserves are needed.

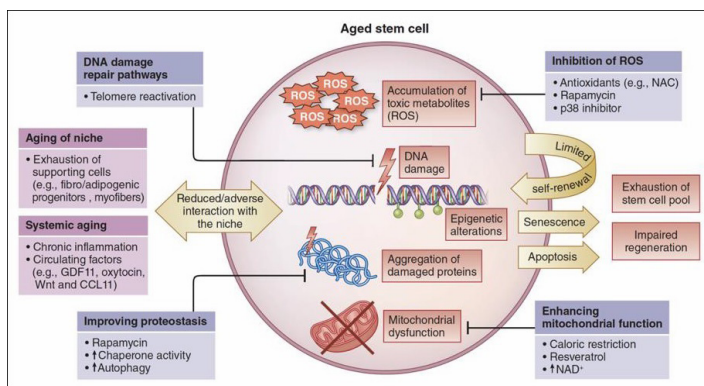
Two more important properties of stem cells are transdifferentiation and transformation. In transdifferentiation, stem cells do not go through their expected, or normal, differentiation pathways, but instead acquire a distinct, new set of properties enabling them to form a new cell line. This ability to form new lines can prove to be very beneficial if a stem cell is ever dislodged from its normal environment and placed in a new environment where other stem cells may be needed. This ability to transdifferentiate is the topic of many research inquiries, including many with inducing stem cells in the myocardium after a heart attack in order to reform essential heart muscle. A second interesting capability of stem cells is their ability to transform. Transformation is “the loss of normal cellular control and is an initial aspect of cancerous tumor formation [3]”.

Transformation is highly related to cancer as most tumors arise from a rapidly dividing population of stem cells. For example, changes to hematopoietic stem cells often lead to leukemia or lymphoma, both characterized by the decrease of a certain kind of blood cell within the body. Transformation does occur with relative infrequency in the body however, suggesting that stem cells status is closely regulated within the body [3]. Stem cell regulation can be further classified as in vitro and in vivo regulation.

In vitro regulation refers to any signals, growth factors or other influencing factors regulating the cell from outside the cell wall or cell membrane, the in the external environment. Changing

environmental demands causes many stem cells to be highly sensitive. An example of one extrinsic regulator is the Notch signaling pathway [3]. The Notch-signaling pathway is highly conserved throughout many eukaryotes and highly present in mammals, across many tissues within multicellular organisms. The Notch-signaling pathway regulates cell determination throughout development and controls adult cell and tissue homeostasis by mediating juxtacrine signaling where both the signal sending and signal receiving cells are changed through ligand-receptor binding [3]. In vivo regulation differs from in vitro, as in vivo signaling pathways are located within the internal environment of the cell.

How do these forms of cell regulation change or control the rate of aging in organisms? As humans age, their body experiences many macroscopic and microscopic changes. These changes often unfortunately lead to an impairment in the functions of many tissues within our system, mostly frequently a decreased response to injury. Stem cells are in our body from conception, as outlined earlier in Figure 1, and throughout our entire life making them highly susceptible to accumulation of cellular damage [4]. This cellular damage to our stem cells is often manifested as noticeable physical impairments in our body. Examples can be our eyes as seen by a decrease in vision causing higher prescriptions, in our nose as our olfactory cells experience a difficulty distinguishing smells and in our brain as a decrease in proliferation of neurogenic progenitor cells leads to a decreased ability to form new memories [4]. These changes are not only occurring within our stem cells, but also within their niches and our body's in vivo and in vitro regulatory pathways. Scientists hope that understanding how stem cells age will be constructive in the development of therapeutic interventions aimed at slowing or reversing these degenerative changes in order to maintain healthy function in aging tissues.



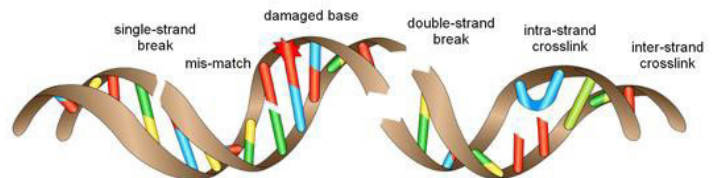
**Figure 2:** Descriptive representation of common pathways contributing to stem cell loss and dysfunction in the aging process. Inside the cell are common physical changes (in red) that occur with aging, while outside the cell (in blue) are possible targets to reverse these mechanisms. Finally, outside the cell (in pink) are common changes occurring within the niche of the stem cell. Figure taken from “Stem cell aging: mechanisms, regulators and therapeutic opportunities” [4].

Stem cells make up such a low percentage of cells within our body, therefore limiting the collection of biochemical changes known, but research is expanding every day. As Figure 2 demonstrates

known changes that occur on the molecular and cellular levels of aging stem cells are: accumulation of toxic metabolites, DNA damage, epigenetic alterations, aggregation of damaged protein, mitochondrial dysfunction, changes within the stem cell niche and changes with the body's regulatory systems as a whole [4].

Accumulations of toxic metabolites come from ever increasing metabolites left in the cell after metabolism. During many metabolic pathways, leaking electrons often escape from the mitochondrial membrane leading to an inhibition of the reactive oxidative species (ROS), a process describing a large amount of reactive molecules and free radicals derived from molecular oxygen [4]. Byproducts during the mitochondrial electron transport of aerobic respiration or by oxidoreductase enzymes and metal catalyzed oxidation have the potential to cause a number of deleterious events within the entirety of the cell. This cellular change can lead to the inhibition of ROS activity in the cell, therefore limiting or ending ATP production in the cell.

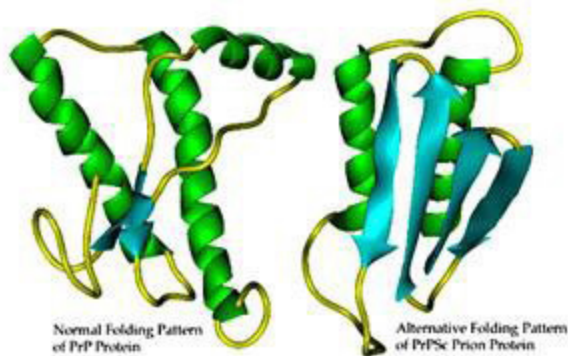
DNA damage also often occurs in aged stem cells as the cell's genome has been exposed to genotoxic particles for a long period of time, often times these stem cells are in a quiescent period, meaning they are inactive and more prone to assault by genotoxic particles. Studies performed in hematopoietic stem cells and muscle stem cells have shown an increasing number of nuclear foci [4]. Nuclear foci are known to be sites of double stranded breaks in the DNA and occur when RAD 51 and RAD 52, enzymes involved in homologous recombination, come in to attempt to seal double strand breaks by unwinding the double strand DNA in those segments [5]. While double stranded breaks are a type of DNA damage, another form of DNA damage also occurs in the cell during the aging process. Research also shows that telomeres are shorter in aged hair follicle stem cells when compared to hair follicle stem cells in humans less than 25 years of age [4]. The average loss of telomeres in vivo is estimated to be about 17 base pairs per year, while some studies document average loss in vitro of up to 1,500 to 2,000 base pairs per year. A third type of DNA damage is changes in our bodies repair mechanism. As many are aware, overuse in our body's muscle causes pain and injury, like overuse in our stem cell's DNA repair mechanisms also cause impairment to their systems. These three changes in the cell's DNA can affect gene regulation, self-renewal properties of stem cells, as well as their differentiation and their ability to undergo cellular apoptosis.



**Figure 3:** Graphical description of possible damages that can occur in the DNA of aging stem cells. Double strand breaks accumulate RAD51 and RAD52, which denoted nuclear foci, a measurable level of DNA damage within the cell. Figure taken from <http://www.bioch.ox.ac.uk/aspsite/index.asp?pageid=892>.

The epigenome refers to DNA modifications, often in DNA binding proteins, which cause variations in gene activity and expression. A common epigenetic change studied is histone modification. Histone modification can present as methylation, acetylation, ubiquitination and phosphorylation of the histone tail which may promote or decrease expression of the genes in those locations. An increasing number of studies conclude that epigenetic regulation is vital in determining stem cell function and therefore indicates alterations occurring in the epigenome associated with aging can impede on cellular processes in aged stem cells [4]. It has been noted that histone acetylation and methylation of the genome changes as the cell ages and the cell begins to transcribe varying RNAs that it once did not, which can delete or overexpress enzymes that stem from those genome sections. Research on ASH-2 trithorax complex, responsible for a trimethylation on the Lysine 4 of Histone H3 has led to the ground breaking observation that the aging epigenome functions like a heritable trait, still capable of modification.

Another form of cellular change occurring in aging stem cells in the increase in aggregation of damaged proteins. Just as our body maintains a normal homeostasis, so too do the proteins within our body maintain a protein homeostasis. Changes in this protein homeostasis often end in variation of protein folding within the tertiary and quaternary biochemical structure of proteins. These changes can lead to protein aggregation, or collecting of these proteins, and an increased damage to the protein can occur if the changes affect the active or allosteric binding sites. Often times changes in structure of these proteins will affect protein functions as they may now be eligible to recognize other active sites or succumb their own active sites to becoming unrecognizable by binding factors. Figure 3 shows an example of a normal folded protein versus an abnormally folded protein. The PrP protein went from a structure of three alpha helices and one beta strand to a structure with two alpha helices and four beta strands. These structure change affect the levels of protein damage and also the proteins ability for destruction and function. The changes occurring within the protein homeostasis can lead to cellular damage and tissue dysfunction as proteins become unable to carry out their jobs within the cell. Proteins are an important determinant in stem cell maintenance, but also changes in metabolite excretion and protein maintenance may also effect stem cell maintenance.



**Figure 4:** This is a graphical description of the normal tertiary folding of the PrP proteins compared to an abnormal graphical description of the

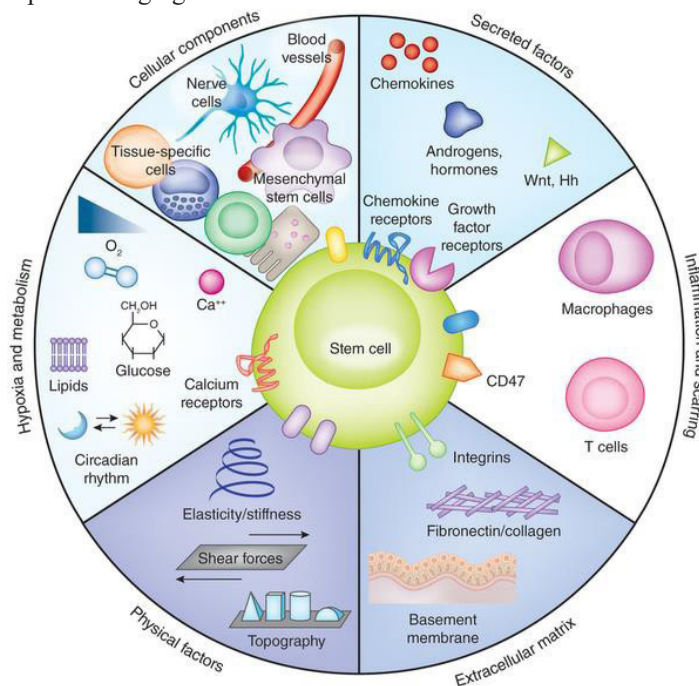
tertiary folding of the PrP protein. Figure taken from *Cells: Daddy or Chips?-An Up-to-Date Review on Ground-Breaking Discoveries in Stem Cell Research* [2].

Mitochondria, often referred to as the powerhouse of the cell, are sites highly susceptible to damage in aging stem cells. Since mitochondria are one of the organelles most highly used in normal function of the cell, they are thought to accumulate mutation at a higher rate than other organelles in the cell. There are two main leading theories as to why mutations in mitochondrial DNA occur. The first theory is that an elevated ROS activity is the principal cause of mitochondrial DNA mutation. Since glycolysis and other metabolic processes occur within the mitochondrial matrix and inner mitochondrial membrane, the mitochondrial DNA is susceptible to damage from free-floating electrons lost in the inner mitochondrial membrane. The second leading theory as to why mitochondrial dysfunction occurs is due to an increasing susceptibility of the gamma subunit if mitochondrial DNA polymerase. This second theory is rooted in minimal research at this time, but has already had some key and important findings sparking further researchers to gain momentum in researching gamma subunit if mitochondrial DNA polymerase.

These six cellular changes occurring in aging stem cells ultimately lead to an ever-aging stem cell with often decreasing the ability of the stem cells to respond to their environment's demands. Experiments on hematopoietic and osteoprogenitor cells reveal large decreases in the population levels of stem cells inversely related to the age of the organism. Experiments have also shown a decrease in the capacity of hematopoietic and osteoprogenitor to form new blood cells and bone, respectively [3]. These finding suggest the functional ability of stem cells declines during development as aging leads to a decrease, or even change, in the ability of stem cells to respond to varying forms of disease and injury. An important note to remember is these changes are occurring within all of the stem cells located in the human body, but will be occurring at different rates according to the type of stem cell. For example, muscle cells in the legs with require the use of more ATP and will tend to have an increase in mitochondrial dysfunction and accumulation of toxic metabolites versus skin cells in legs may have an increase in DNA damage due to exposure to the ultraviolet radiation from the sun.

All of these changes added together lead to a decrease in functionality and decrease in the number of stem cells in the stem cell pool available for use within the body. Stem cells often choose one of two fates when the aging processes increases: apoptosis or senescence. Apoptosis, or programmed cell death, occurs when the cell is no longer viable due to internal damage. Senescence occurs when the stem cell remains alive but it is unable to differentiate further or enter or exit the cell cycle [5]. It is unknown why the cell would choose one fate over another, but there has been a tissue-specific difference noted between certain tissues holding on to an increased number of senescence stem cells compared to another tissue. For example, hematopoietic stem cells generally increase in number with age, but their progeny (red blood cells, white

blood cells, etc.) do not produce at any more of a rapid rate, thus suggesting that although more stem cells are present, these stem cells may be in a senescence state with “reduced hematopoietic reconstituting activity and skewed differentiation potential” [5]. Stem cell exhaust can also be driven from a combination of these internal chemical changes and an imbalance between stem cell quiescence and proliferation. A correct balance of these divisions is essential due to excessive proliferation causing a decrease in cell cycle regulation or changes within internal levels of ROS. An increased demand for replacement of cells within mature tissues may also decrease the regenerative capacity of cells by increasing replicative aging.



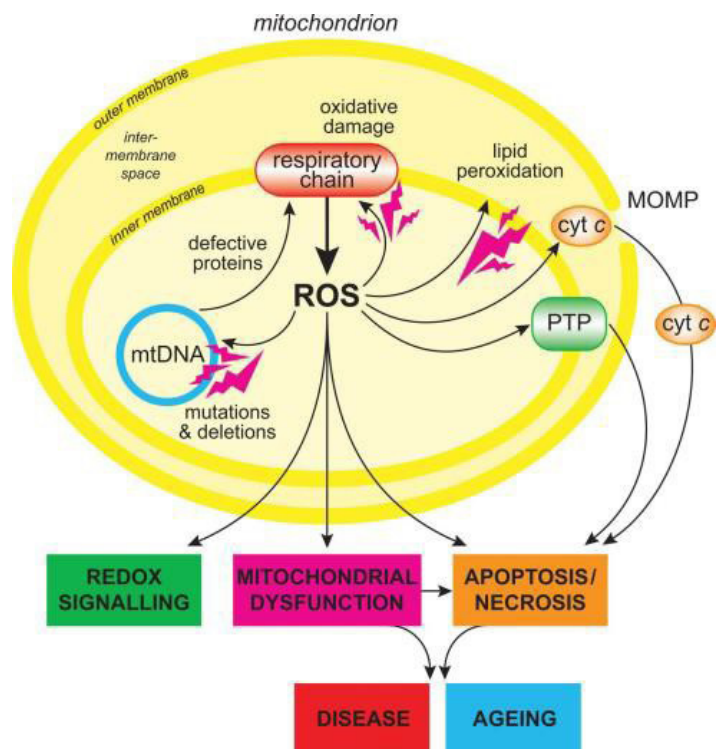
**Figure 5:** A graphical description of the stem cell niche, including physical factors, hypoxia and metabolic factors, the extracellular matrix, proteins involved in inflammation and scarring, and cellular components from tissue-specific cells and secreted factors. Figure taken from “Modulating the stem cell niche for tissue regeneration”[7].

Changes not only occur within the stem cell itself, but also within the environment directly outside of the cell, called the stem cell niche. The stem cell niche is often referred to as a local microenvironment. Figure 5 gives an accurate description of all of the varying factors, proteins, signals and more that are included within the stem cell niche. The stem cell niche is primarily responsible for transmitting signals from the external environment to the stem cell, often controlling if the cell will proliferate, divide, differentiate or so on. The signals from the niche may trigger deregulation of stem cells by decreasing the level of maintenance they are given. Niches for immune cells can also serve as critical regulators of satellite cell function during muscle regeneration, often coordinating inflammatory responses to prevent their premature differentiation. Age-related changes in the stem cell niches may trigger the end of a cell line or premature differentiation.

Aging also occurs within the systemic circulating factors of the

body. Chronic inflammation of mediators or accumulation of stem cells in the senescence state may contribute to dysfunction associated with stem cell aging. For example, as mice age an elevation in transforming growth factor (TGF)- $\beta$ , a growth factor known to impede regeneration and satellite cell proliferation, has been noted. Moving into how this research can be used in the treatment of reversing these six changes occurring within stem cells and their niche will be the next step in this paper. Creating drugs, proteins or diets aimed at targeting each one of these pathways is a realistic possibility to achieving the goal of improving stem cell therapy.

To target the accumulation of toxic metabolites within our cells, researchers are using a known ROS scavenger. ROS scavengers, often referred to as antioxidants, are an eliminator system found within the mitochondria to eliminate toxic damaged caused by ROS intermediates and metabolites. Research has been performed with the known ROS scavenger, antioxidant N acetyl-l-cysteine (NAC) [5]. NAC has previously been used to ameliorate ROS effects within somatic cells, so the jump to use in treatment of stem cells seems natural. Studies show that NAC has produced successful decrease in the level of ROS in myogenic stem cells in skeletal muscle, both in vivo and in vitro. Research within these cells has proven to have logarithmic therapeutic opportunities as treatment within these cells can be used to cure many lifelong and generational diseases, such as arthritis and joint deterioration [5]. These studies with NAC show that reducing ROS levels can reverse aging phenotypes caused by an uncontrolled accumulation of ROS. Research is also showing that more information is needed on how the endogenous source causes the ROS activity to ensure NAC is affecting ROS rather than only controlling the effects caused from the accumulation of toxic metabolites.



**Figure 6:** Graphical description of a mitochondrion's metabolic processes with detailed description of ROS activity leading to mitochondrial damage within the cell. Figure taken from "How mitochondria produce reactive oxygen species" [6].

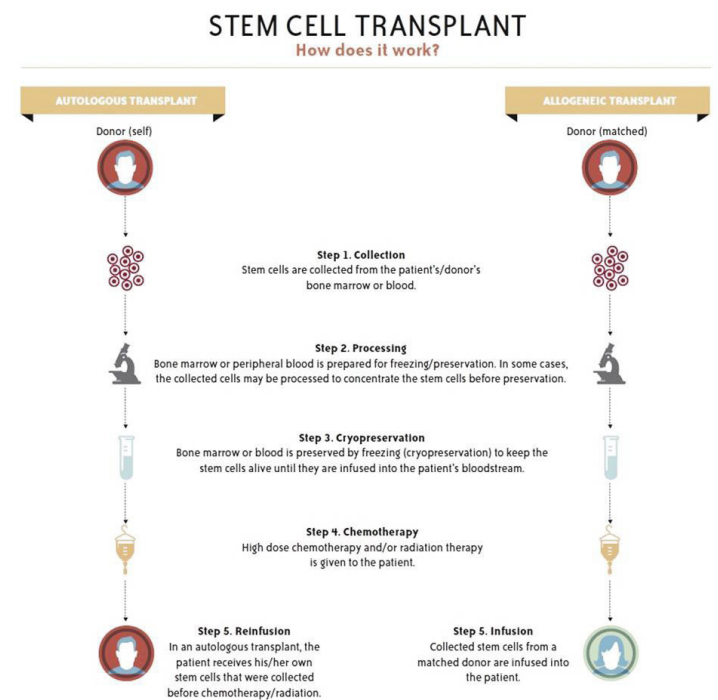
In an effort to decrease age-related DNA damage within stem cells, research is now aimed at increasing the DNA repair pathways already in place in the cell or by adding new DNA repair pathways to the cells. It is reasonable to assume that increasing DNA repair activity will slow or prevent accumulation of age-related defects in stem cells [5]. A reasonable process to increase DNA repair pathways is to identify master regulator genes in charge of synthesizing inputs from multiple DDR pathways to an effort to exert sufficient needed support in the correct tissues and at the appropriate levels and functionality, in conjunction with additional signaling processes. This exact process has been used as means for telomerase reactivation. Telomerase is responsible for regenerating telomeres, the ends or shoelaces of DNA that do not encode for genes, in an attempt to protect the coding gene of the genome from degradation during replication and transcription. When telomerase was reactivated in stem cells, the nuclear foci, spots of double stranded DNA repair, levels had decreased when stained with 53BP1, a DDR protein [5]. A note about increasing the telomeric pathway within cells is that cancer cells often used this pathway when they are attempting to overcome replicative senescence. Within cancer cells reverse telomerase overexpression has been shown to induce an ever-increasing variety of malignancies. Since the mechanism of how these malignancies occur is not completely known, researchers are timid of using this method for further treatment of stem cells in order to avoid the possibility of inducing cancer.

Epigenome pharmacologic treatment that alters histone modification, such as acetylation and methylation, may show an increase or decrease in the longevity of aging of the progeroid mouse models. Their studies are aimed at reprogramming aged somatic cells (for example a skin cell) into an induced pluripotent stem cell, or a cell similar to an induced pluripotent stem cell. This re-differentiation from somatic to stem cell could be useful in resetting the memory of aged somatic cells in terms of their epigenome. There may be more clinically viable options to alter or reverse the epigenome of aging stem cells, but further analysis of epigenetic signaling of stem cells can help to find more viable options.

Improving protein homeostasis has not yet had been the direct target of research through either stimulation of autophagy or proteasome activity. There is evidence from mice that have been genetically manipulated to preserve chaperone-mediated autophagy shown to have an increased response to stress, therefore preserving more functional organs with age [5]. Chaperone-mediated autophagies are involved in the selection of specific substrates that improve cellular and protein homeostasis throughout the entirety of the stress reaction. Stress response is often linked to the old saying 'what doesn't kill you makes you stronger'. As cells are placed in increasing stress environments, the level of their stress response

proteins being to increase as does their antioxidant activity. The increase of these two factors allows the cell to undergo a greater stress level than it would have been able to endure prior to exposure from the first stress. So if the level of stress does not kill the cell initially, the will become stronger and able to endure more, thus relating itself to the famous saying.

Enhanced mitochondrial function can lead to an increase in the function of stem cells, along with their self-renewal and differentiation properties. As frequently studied throughout metabolism laboratories, calorie restricted diets often induce minimal stress into the environment by pushing the cell to work harder than normal for its food. Short-term use of these calorie-restricted diets is widely employed as an intervention to decrease the changes that are often associated with aging phenotypes. Even long-term use of calorie-restricted diets has been shown to delay the onset of these age-related changes in stem cells. In the terms of brain stem cell function, calorie restricted diets proved to increase the expression of brain-derived neurotrophic factor and therefore reducing the mTOR complex 1. Further research into how mitochondrial dysfunction is caused shows a proportional relationship between low levels of NAD<sup>+</sup> and mitochondrial decay. These changes are most common within myogenic stem cells, or stem cells often located in the skeletal muscle.



**Figure 7:** Depiction of the two kinds of stem cell transplants, autologous and allogeneic, with respect to cancer therapy. Note whether the stem cells are coming from the donor or an outside source, the procedure for a stem cell transplant is conserved throughout the process. Figure taken from Winthrop P. Rockefeller Cancer Institute at <http://cancer.uams.edu/bone-marrowtransplant/>.

Replenishing active stem cells with stem cells pools already within the body could be another therapeutic effect for decreasing the effect of stem cell aging within the body. This replenishment

could occur through simple injection of stem cells into injured or diminished tissues, called a stem cell transplant [6]. Famous studies with convincing results have already proved this process is a viable option, as athletes like Tiger Woods and even some NFL athletes have had spectacular recovery and regeneration. These replacements usually occur from an unaffected donor or autologous cells. Figure 7 explains more details of stem cell transplants including harvesting, processing, cryopreservation, chemotherapy and either reinfusion or infusion if taken from an outside donor. For example, transplantation with hematopoietic stem cells, originally taken from complete bone marrow, peripheral blood or umbilical cord blood can be closely matched and allowed to proliferate creating many new blood cells, including white blood cells, red blood cells, platelets and much more [7]. Increasing technology for infusion has allowed more and more patients to receive their own stem cells, decreasing the chance for immune response when cells are transplanted from an outside source. Another option for replenishing the stem cell pool is to reactivate the stem cells in senescence or a quiescent state. These techniques must be able to maintain the balance of stem cell self-renewal and differentiation.

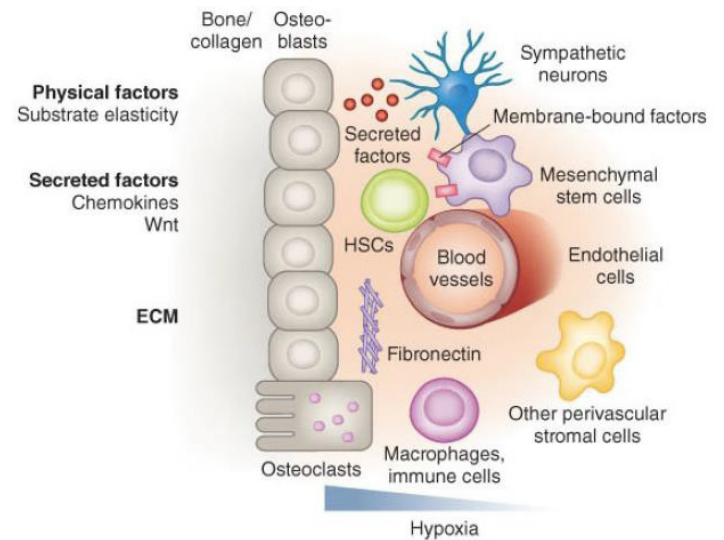
The famous experiment of surgically sewing older mice to younger mice showed that the aging of stem cells is directly related to environment in which the stem cells are placed. Stem cells from the older mice were enhanced in neurogenesis and re-myelination of myelin sheaths on neurons, both neurogenic properties famous for decreasing with age [7]. Research on activating the environment in order to activate the stem cells located within is currently being performed.

Many barriers to these treatments include cost and lack of complete knowledge at this time. Costs of raising animals into ‘old age’, approximately equal to 25 years old in humans, for use in testing laboratories is expensive [5]. There are also large differences within the stroma and microenvironments of tissue-specific stem cells, causing a large variation in possible treatments and also undoubtedly translate into differences in the way these tissue-specific stem cells are aging. Stem cells differ in their number of divisions throughout their life, for example the rapidly dividing stem cells located in the gut versus the slowly dividing stem cells located in the spinal cord. A better understanding of how these mechanisms maintain themselves is needed and may provide valuable insight onto the stem cell aging process [7]. Drug design specifically targeting stem cells is also difficult. For example, antibiotics often have many pathways they can target due to the large differences between prokaryotes and eukaryotes, while there are only minimal differences between somatic cells and stem cells. This detail creates a barrier to design where researchers are designing drugs, diets and proteins only affecting correct cells within the body.

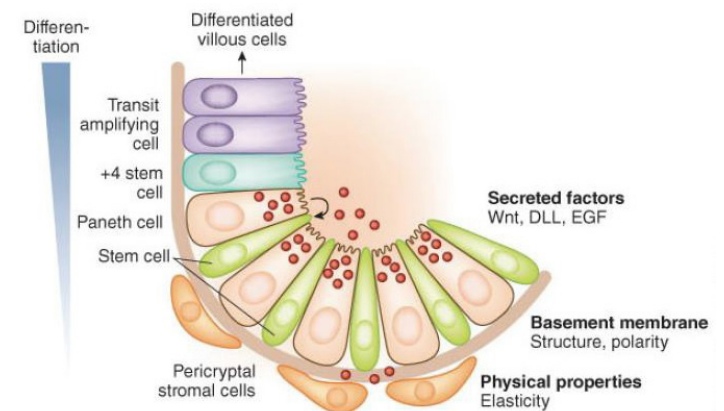
Moving into how the stem cell niche can be modulated for tissue regeneration is the next section in this review, but is also a growing field within stem cell community as whole.

In search for today’s fountain of youth, activating stem cell niches

in order to find viable options for reactivating senescence or quiescent stem cells may be the most viable option. Changes not only occur within the cell itself, but also within the environment directly outside of the cell, called the stem cell niche, a local microenvironment [7]. The stem cell niche is primary responsible for transmitting signals from the external environment to the stem cell, often controlling if the cell will proliferate, divide, differentiate or so on.



**Figure 8:** Representative description of a discrete niche supporting a stem cell in the epidermal layers. Note the large variation on shapes and sizes of factors affecting this stem cell niche. Figure taken from “Modulating the stem cell niche for tissue regeneration” [7].



**Figure 9:** Descriptive representation of a discrete niche supporting an intestinal stem cells. Note the large number of varying cells when compared to the epidermal stem cell. Figure taken from “Modulating the stem cell niche for tissue regeneration” [7].

Figure 7 and Figure 8 show drastic differences in the environment surrounding epidermal and intestinal stem cells. It may be inferred that stem cells located within the skin and intestine require variations in their stem cell niche, due to their very different functions within the body. Epidermal cells must be able to protect their DNA from large amounts of ultraviolet radiation often caused from the sun, while cells within the intestine must be able to protect themselves from often very acidic to often very basic conditions.

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The signals from the niche may trigger deregulation of stem cells by decreasing the level of maintenance they are given. Niches for immune cells serve as critical regulators of satellite cell function during muscle regeneration, often coordinating inflammatory responses to prevent their premature differentiation. Age-related changes in the stem cell niches can trigger the end of a cell line or premature differentiation. Schofield was the first to hypothesize in 1978 that the self-renewal ability of stem cells is directly related to their niche, the environment maintained by neighboring, non-HSC cells [7]. Key factors now known about the niche are; interactions among stem cells and those cells neighboring, factors secreted, current inflammation and scarring, the extracellular matrix, differing physical parameters (i.e. prolonged or acute stress or stiffness in the tissue), and varying signals from the environment (including hypoxia).

An increasing number of studies are aimed at describing the important of the stem cell niche for the stem cell to modulate behavior and retain their stem cell specific qualities. Describing the microenvironment, or niche, of hematopoietic stem cells can prove to be very important. Tissues surrounding the heart myocardium will have different needs than the tissues in the spinal cord and tissues in the bone marrow. Since there're many types of cells that perform many different functions throughout our body, it is reasonable to expect the niche, or microenvironment around our stem cells, to also be varying according to the tissue. Skeletal muscle in the quadriceps will likely have specific factors that allow metabolic pathways to occur at a faster rate than cells not requiring as much ATP located in the epidermis.

In many adult tissues located throughout the body the stem cell niche contains various cells all with varying functions. Cells of note are osteoblastic, vascular, neural, while macrophages and other immune cells are also present. Stem cells from the same tissue, when located next to each other, contain complex bidirectional signaling, often referred to as cell-to-cell contact. Many membrane-bound factors bind to the surface of stem cells in the attempt to regulate their self-fate, the stem cell's self-renewal properties and polarity of the stem cell. Cells involved in fighting off pathogens, often referred to as immunological cells provide dynamic regulation of their niche environment due to a property called immune privilege. There are also proteins in the extracellular matrix that are critical for maintenance of niche. These proteins provide constructive signals, often times through ligand interaction. Shape and stiffness, as well as blood flow, also affect stem cell maintenance and their self-renewal and differentiation properties. Stem cells located in muscle tissues must also be able to adapt to altered environmental characteristics, for example stem cells must be able to adapt to hypoxia during long aerobic workouts.

While these differing stem cell niches cause an increase in the thirst for knowledge about their differences, there are also many barriers to research. There are also large differences within the stroma and microenvironments of tissue-specific stem cells, causing a large variation in possible treatments and also undoubtedly translate into differences in the way these tissue specific stem cells are aging.

Stem cells differ greatly in the number of divisions throughout their life, for example the rapidly dividing stem cells located in the gut versus the slowly dividing stem cells located in the spinal cord. A better understanding of how these mechanisms maintain themselves is needed and may provide valuable insight onto the stem cell aging process [7]. Drug design, specifically targeting stem cells, is also difficult. For example, antibiotics often have many pathways they can target due to the large differences between prokaryotes and eukaryotes, while there are only minimal differences between somatic cells and stem cells.

## Summary & Conclusions

Stem cells can prove to be a very effective tool in the treatment of many age-related diseases today. These cells differ from other cells in the body, often referred to as somatic cells, due to their ability to, first, self-renew. The second main function of stem cells is their ability to differentiate and give rise to cells with more specialized functions than their self. As stem cells age, they are often subjected to many internal and external stressors, causing harm to many different parts of the stem cell [7]. Known changes that occur on the molecular and cellular levels of aging stem cells are: accumulation of toxic metabolites, DNA damage, epigenetic alterations, aggregation of damaged protein, mitochondrial dysfunction, changes within the stem cell niche and changes with the body's regulatory systems as a whole.

These main changes occurring at the biochemical level within stem cells have been an area of intense study in the hope that reversing or stopping these changes from occurring can reverse many of the effects aging has on the stem cell. To treat the accumulation of toxic metabolites, caused by leaky electrons within the inner mitochondrial membrane during metabolic pathways, the antioxidant N-acetyl-l-cysteine has been added to the cell. Antioxidants are known for their ability to scavenge to ROS damage and eliminate that damage, often called ROS scavengers. Treatment with antioxidant N-acetyl-l-cysteine has positive results on decrease of ROS damage, but it does remain unclear if these treatments are preventing ROS damage or fixing the effects of the damage.

For DNA damage, mTERC was added to the stem cells to treat both the decreasing telomere length and the decreasing ability of the cell's DNA repair mechanisms. mTERC is a telomerase reverse transcriptase subunit, which helps to transcribe telomeres during each round of replication in order to ensure coding genes are not being damaged during replication. To reverse the effects of damaged proteins or changes in protein homeostasis, no formal treatment has been proposed but coinciding research has shown that pharmaceutical inhibition of mTOR by rapamycin has been promising to restore many of the self-renewal properties known to be held by hematopoietic stem cells. Targeting mitochondrial damage is also another area of fertile research as the cause of mitochondrial dysfunction in aged stem cells does not have a consensus conclusion at this moment. However, there have been studies showing that short-term and long-term use of a calorie restricted diet often delays or decreases the effects caused by aging



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on the mitochondria. Treatments aimed at reversing epigenetic modifications that occur throughout aging also need more research and knowledge before coming to fruition. Finally, alterations to the stem cell niche have also been proven to aid in maintenance of the self-renewal properties, although more research is needed according to the various needs of each tissue-specific stem cell, as it has been noted the environment around stem cells drastically changes according to the tissue-specific type of stem cell.

In conclusion, reversing these 6 noted aged-related changes occurring within the stem cell can be a very successful way to decrease the overall effects of aging within the human body, however, more research is needed before this idea will be able to come to fruition. Many of the mechanisms and systems causing these changes are not completely understood at this moment, but once these ideas are flushed out, tools to reverse the effects of aging will have the opportunity to be better derived. Research is increasing at a logarithmic rate on stem cells and the stem cell niche, with this paper providing many possible avenues for further research. The idea of a young stem cell pool capable of self-renewal and differentiation for longer periods of time is a reasonable idea considering facts and knowledge presented in this review paper.

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