Title

Advanced Techniques for Stem Cell Therapies: A Dual Approach to Cell Senescence and Stress-Induced Proliferation

Journal

Undergraduate Laboratory at Berkeley, issue 2024-2025

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Publication Date

2025-10-01

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Abstract

This literature review aims to identify a combined avenue to prevent cell aging and stress-induced proliferation in stem cells. Cellular senescence is a natural process that prevents cells from replicating, while stress-induced proliferation is uncontrolled cell division. Although these processes appear to be opposite, they both must be carefully regulated when creating stem cell therapies; the stressor of administering stem cell therapy can deregulate both pathways, which increases the risk of diseases such as cancer. This paper examines how two major techniques, CRISPR[1]-mediated gene editing and SASCs[2], can be used to regulate cellular senescence and cell aging. CRISPR mediation has been proven to extend stem cell viability by inhibiting telomerase activation and regulating stress-response pathways. SASCs act as an alternative to embryonic stem cells in therapies; their ability to maintain redox balance through an antioxidant system allows them to serve as a more hardy and renewable stem cell source. This paper proposes a novel avenue of research that involves integrating both techniques into future stem cell therapy research, thereby addressing both cell aging and stress-induced proliferation. Future research should explore and refine a combination of these techniques and evaluate their efficacy to complement existing stem cell technologies.

Keywords: CRISPR, SASC, cell senescence, cell proliferation

[1] CRISPR: Clustered regularly interspaced short palindromic repeats
[2] SASC: Synthetic artificial stem cell

Introduction

Cell senescence is a natural process of the cell cycle that enables organisms to regulate dysfunctional cell proliferation and prevent the expression of oncogenic genetic properties. In contrast to cellular aging, cell senescence is one of multiple ways cell aging occurs, as it is a stress-induced process resulting in a cell entering a stage of arrested proliferation, with no known physical means of allowing such a cell to reenter the cell cycle (Campisi, 2012). Cell senescence regulates, to some degree, the proliferation of cancer cells and the breakdown of dysfunctional cells for tissue repair. However, it can also cause certain age-related diseases and interfere with organ function, posing key obstacles to the development of stem cell treatments (Zhang et al., 2022). Novel research findings have identified metabolic regulation as a key factor dictating cell senescence, offering the possibility of rescuing stem cells from cell senescence through metabolic means. Cellular reprogramming and growth factor signaling have additionally illustrated technological means of allowing stem cells to persist in their intended environments, increasing the effectiveness of stem cells in regenerative medicine (Beltrami et al., 2011).

Abnormal proliferation within cells is prevalent in the presence of stressors, such as inflammatory cytokines, stress hormones, and reactive oxygen species. With the utilization of stem cell technology and Clustered Regularly-Interspaced Short Palindromic Repeats (CRISPR) gene-editing techniques, the genes of stress-signaling pathways can be targeted to increase cellular stress tolerance. The paper recognizes the importance of such advancements and encourages further research in this area, particularly in applying them towards cancer therapies. Inhibiting stress-induced proliferation with CRISPR can regulate the stress-response mechanisms that oncogenic cells often depend on to survive and proliferate under harsh conditions, decreasing oncogenic expression. One recent CRISPR advancement has identified suppressors of

endoplasmic reticulum stress-induced apoptosis (Panganiban et al., 2019). Biomarkers like these suppressors and disruptions in signaling pathways are clues that indicate stressors and their effects. Researchers can target a much shorter spectrum of factors that induce stress on cells through biomarkers. However, most research on CRISPR and stress response has been performed on an abiotic scale in agricultural crop enhancement; more research is needed to fully comprehend the biological mechanisms behind stress response. By understanding stem cell activity in places such as chronic stress conditions, treatments can be made to increase cell tolerance. The uncontrolled division of cells is a key factor in cancer development, and treatments that attempt to target cellular proliferation should also target the stress conditions that trigger it for more effective treatment results.

Stem cell therapies have great potential in regenerative medicine, a branch of medicine focused on regenerating cells and tissues to heal damage from degenerative diseases. While technologies like CRISPR are becoming increasingly more prevalent in disease treatment, the ability of stem cells to differentiate allows for the additional possibility of prior disease prevention. This holds special promise in preventing genetic diseases in children; advanced stem cell therapies could replace disease-inducing mutations in stem cells that would otherwise differentiate into tissues carrying the mutation throughout development. Although such research is in its early stages, base editing has shown potential for stem cell therapies in disease prevention when administered early in development. Essentially, by focusing on stem cells, researchers choose to focus on treating risk factors that could catalyze the development or furtherment of the disease rather than the disease-ridden cells themselves.

CRISPR is a groundbreaking gene-editing technology that has transformed molecular biology. Originally discovered as a component of the adaptive immune system in prokaryotes,

CRISPR, along with the CRISPR-associated (Cas) proteins, enables precise genetic modifications by targeting specific DNA sequences. The simplicity and specificity of CRISPR/Cas9 have made it an essential tool in biomedical research, agriculture, and therapeutic development (Lino et.al, 2018). As such, CRISPR has become a powerful tool for modifying stem cells, providing new opportunities in regenerative medicine, disease modeling, and drug discovery. One of the most promising applications of CRISPR in stem cell research is the genetic correction of disease-causing mutations. In addition to correcting mutations, CRISPR can be used to create disease models by introducing specific genetic mutations into stem cells. These models allow researchers to study the mechanisms of genetic diseases, such as cystic fibrosis or sickle cell anemia, and test potential treatments within a controlled environment (Lino et al., 2018).

CRISPR-Cas9 has extensive potential to treat a wide range of diseases, such as cancer. However, since CRISPR is conducted *in vivo*, within a living organism, the potential threat of Cas9 cutting the wrong sequence of DNA is a significant risk, posing a major obstacle in using CRISPR-Cas9 for therapeutic purposes. Fortunately, scientists have developed a way around this situation with the introduction of High Fidelity (HiFi) Cas9 (Vakulskas et al., 2018): this Cas9 mutant was specifically designed to reduce the off-target effects that CRISPR-Cas9 previously possessed. In addition to this, there are several other applications in which CRISPR can be used. Methods include CRISPR interference and CRISPR activation, using CRISPR to repair damaged DNA and specifically target cancerous stem cells.

CRISPR also plays a role in large-scale drug screening by enabling the modification of stem cells for testing drug response. This approach accelerates the discovery of new treatments by allowing researchers to evaluate how genetic modifications influence drug effectiveness and

toxicity (Lino et al., 2018). Despite these promising applications, challenges remain in efficiently delivering CRISPR to stem cells and ensuring the safety of gene edits, particularly for clinical use. As delivery technologies advance, CRISPR is expected to play a pivotal role in the future of stem cell therapies and personalized medicine (Lino et al., 2018).

While much research has been conducted on the topic of cell senescence and stress-induced proliferation, as well as the current technological means of advancing stem cell compatibility, there is a dearth of research focusing on applications of these technologies to achieve the goals of the former topic. By using technological solutions to rescue cells from senescence and inhibit stress-induced proliferation, the range of diseases for which stem cell treatments are appropriate significantly increases. Although cell senescence and stress-induced proliferation are nearly opposite in nature, both phenomena impact one another: the absence of one largely leads to adverse conditions from the other. By tackling both sides of the issue with a combined treatment, the expansion of stem cell treatments will bring with it a greater possibility to advance human health.

Methodology

This research paper revolves around the question of how various stem cell technologies can be used to address cell aging and stress-induced proliferation. With the development of such technology, methods such as synthetic artificial stem cells (SASCs) and clustered regularly interspaced short palindromic repeats (CRISPR) can be vital for maintaining a healthy immune system. This paper includes an overview of these methods, their applications, and possible implications for future research and developments in the field of regenerative medicine. The paper focuses on various techniques used to modify stem cells to either reduce cell aging or inhibit stress-induced proliferation. Experiments performed to identify each technique are presented, and their outcomes are discussed in each section. Similarities in the techniques are also analyzed to identify a novel research avenue for a combined treatment. Finally, possible applications of such a combined treatment are discussed.

The paper primarily utilized search engines and databases to access past research conducted on the relevant topics of this discussion. Google Scholar, PubMed Central, and bioRxiv were the primary search engines used in this process. It is important to note that some articles in this literature review were accessed using the UC Berkeley institutional authorization system. Keywords inputted into the search engines to identify potential papers include: “stem cell”, “cell proliferation”, “cell aging”, “CRISPR”, “cancer therapies”, “stress-induced cell”, “synthetic artificial stem cell”, etc. Keywords were both used by themselves and in combination with others to find the most relevant, accurate, and peer-reviewed articles.

In writing this scientific review paper, numerous inclusion and exclusion criteria were applied to ensure research with accuracy and reliability. The primary metrics used in selecting sources included their specificity to stem cell treatments and the broadness of the research’s

applications. Numerous sources that were initially considered for analysis were later excluded, as their findings only applied to a specific disease and lacked generalizability due to the niche applications presented. Additionally, a recency requirement was implemented, particularly due to the topic of stem cell regenerative medicine being a somewhat recent point of discussion among the scientific community. Papers published in the last 15 years were considered for review, while sources beyond this date were considered only for reference and possible comparison if conflicting findings arise. All articles were peer-reviewed and published on literary and scientific databases, such as Google Scholar and PubMed. Throughout this process, rigorous reflection on the available literature was conducted, and academic integrity was prioritized to present accurate and applicable research.

Results

Cellular Senescence

Cellular senescence is a vital regulator of organismal functions in controlling malignant growth, but it also presents challenges in applications of regenerative medicine by interfering with cellular repair (Beltrami et al., 2011). With much of stem cell research focused on alleviating pre-existing medical conditions through the unique undifferentiated properties of stem cells, integrating more renewable technologies like synthetic artificial stem cells (SASCs) and CRISPR alterations will become important in sustainability and broadening the range of treatment options. Cellular treatments are fundamentally limited by the inherent upper limit on cellular differentiation, known conceptually as the Hayflick limit, which limits cells to proliferate to a maximum of 50 +/- 10 generations (Hayflick et al., 1961). However, in previous studies, human embryonic stem cells (hESCs) have already displayed promising traits for the field of regenerative medicine, such as nearly unlimited proliferation through self-renewal when grown as a culture as well as relatively stable genomic structure across generations (Zeng et al., 2007), albeit mutations in such cultures continue to raise concerns on their practical application.

Despite the apparent challenges, stem cell technology has had several advancements through the use of gene-editing techniques, which is the main focus of this review. In an example study concerning the proliferation of neural stem cells, specific pathways such as Mysm1 displayed profound changes to brain structure when knockout was induced in aged organisms, revitalizing cellular repair through neurogenesis, but induced apoptosis when occurring in normal individuals (Xu et al., 2024). This suggests that greater specificity in the type and conditions in which gene editing, such as gene knockout, is induced may allow for greater applications in instances where such a procedure may be detrimental in other contexts.

CRISPR, Cas9-mediated gene corrections, SASCs, and other augmentative *in vitro* technologies present greater potential for stem cell advancement. Cell senescence, as previously defined, is primarily a stress-induced response, whether it be beneficial in the event of abnormal proliferation or premature in commencing the aging process. Studies on specific genetic diseases have shown applications for stem cells; mesenchymal stem cells have been used to correct Cockayne syndrome by generating differentiated cells that are corrected of pathogenic mutations via targeted gene editing techniques (Wang et al., 2020). Other studies have focused on signal inhibiting mechanisms such as Wnt/β-catenin signaling inhibitor DKK1 or β-catenin siRNA (Zhang et al., 2011) to observe mesenchymal stem cells' ability to reverse senescence. These studies suggest possibilities of using these gene-editing technologies to alter genetic composition and expression within stem cells to target cell senescence, allowing for more profound treatments by taking advantage of stem cell renewal and differentiation.

CRISPR Techniques for Cellular Senescence

CRISPR has become an increasingly relevant practice in current scientific research, especially in epigenome editing. This can be applied to cell aging to restore or reverse the aging process, essentially extending the cell's lifespan. The TERT (Telomerase Reverse Transcriptase) gene is a crucial factor that can be used for this process to become successful. With the guidance of CRISPR, the TERT gene targets the telomerase enzyme, which helps maintain cell lifespan by extending chromosomal telomeres through nucleotide addition (Brane et al., 2019). Telomeres, also regarded as "caps", are DNA sequences that act as a protective structure at the ends of chromosomes. As cell division occurs, telomeres get shorter and shorter. Once they are too short, the cell loses the ability to divide and have a chance of dying off. This is where CRISPR and the TERT gene play their role: as TERT targets the telomerase, it sends a message to the enzyme,

essentially programming it to repair the telomeres by adding additional DNA sequences. This progression leads to the lengthening of telomeres, which in turn extends the lifespan of the cell (Brane et al., 2019). By applying gene-editing technology like CRISPR to stem cell transplantation, one such use could be in stimulating expression of the TERT gene, allowing for possible rescue of senescent cells from their non-proliferating cycle (Porika et al., 2022).

CRISPR has also shown potential in reversing cell aging through applications in Yamanaka factor activation. The Yamanka factors are a group of four transcription factors that are highly expressed in embryonic stem cells: Oct4, Sox2, Klf4, and c-Myc. These transcription factors have the potential to reverse cell aging by targeting specific DNA strands to control gene expression. Prior studies exhibit the restorative potential of the Yamanaka factors in restoring vision to mice (Lu et al., 2020). Scientists used three of the Yamanaka factors (Oct4, Sox2, Klf4) to exhibit this restoration. This gene expression could be controlled with the use of a man-made virus, allowing for direct control in targeted areas. In restoring vision, the Yamanaka factors prove to have higher beneficial effects in older mice than middle-aged mice, further implying variability between organism age that these transcription factors can produce in cellular aging (Lu et al., 2020). While these studies did not directly use CRISPR, the technique can be applied in a similar fashion. Guiding the desired Yamanka factors with CRISPR allows for specifically targeted areas to be reached in several areas of the human body. As proven with the mice study, the Yamanaka transcription factors have extensive potential; however, risks are posed when it comes to their proliferation, as the Yamanaka factors rely on extensive proliferation to function. While CRISPR technology to target Yamanaka transcription factors is effective in reversing cell senescence, it must be carefully considered in combination treatments that also desire the reduction of cell proliferation.

Additionally, the *p53* gene was found to be a significant regulator of cell growth in response to oxidative stress. Mutations in the *p53* gene, whether loss or inactivation, are prevalent in cancer because of the loss of cell-cycle arrest or apoptosis after oxidative stress (Reisman et al., 2012). This, however, can be beneficial in the case of promoting cell proliferation for cells undergoing senescence. Similar to the *Keap1*-Nrf2 relationship (refer to Section: CRISPR Techniques for Stress-Induced Proliferation), the *p53* tumor suppressor's activity is inhibited by the Mdm2 protein that acts as an E3 ubiquitin ligase. E3 ligases recognize and bind to particular proteins in the ubiquitination pathway, which triggers cell division and regulates the cell cycle. When a phosphate group is added to the Mdm2 protein at a serine residue site (this process is called phosphorylation), it changes the way the Mdm2 protein binds to and interacts with the *p53* tumor suppressor. To observe the role of the Mdm2 protein when not phosphorylated, Chibaya et al. used CRISPR on mice to generate a mutant Mdm2 protein that cannot be phosphorylated at serine residue 183 (Ser183). Through their observation of the mouse embryonic fibroblasts (MEFs), the researchers found that Akt phosphorylation of the Mdm2 at Ser183 decreased *p53* levels, while the mutant Mdm2, not phosphorylated at Ser183, increased *p53* levels. MDA amounts showed that MEFs did not undergo senescence in response to oxidative stress (Chibaya et al., 2021). Thus, the phosphorylation of the Mdm2 protein is beneficial and encouraged in future cell senescence studies. In this case, CRISPR was utilized to provide the significance of phosphorylation for properly regulating oxidative stress response while preventing senescence.

Synthetic Artificial Stem Cell Techniques for Cellular Senescence

Different stem cell types have curative effects on various bodily functions. Embryonic stem cells (ESCs) show improvement in ovarian function, while induced pluripotent stem cells

(iPSCs) are primarily effective on ventricular function (Chang et al., 2022). Artificial stem cells are a beneficial tool that can be altered to fit specific treatment sites (Chang et al., 2022). Using stem cells to exert protection against adult degenerative diseases illustrates the significance of being able to tactically manipulate stem cells to counter cell aging through preventative treatments.

Preventative stem cell transplantation during the most primitive neonatal stage exerts optimal protection against both neonatal and adult diseases. Once such method supplies stem cells through the umbilical cord blood, which has been tapped into as an abundant source for stem cell banking (Sanberg et al., 2014). Delays in cord clamping increase stem cell supply to the baby, providing immediate benefits if neonatal disease is indicated. Transplanting stem cells from mother to baby may be the most non-invasive therapeutic science for preventing mortality associated with neonatal and adult diseases (Sanberg et al., 2014). The process of preventative stem cell transplantation allows for more stable cell aging of organs in the long run, helping prevent age-related disease by allowing greater flux of natural stem cells through the umbilical cord. Preventative stem cell transplantation reduces the need for invasive treatments by increasing the presence of natural stem cells beneficial for the body earlier in life, which reduces the risk of high rates of cell aging and mortality.

Stem cells of different types can be utilized via standard intracavernous injections into a host to document the effects of artificial stem cells in improving organ function (Argiolas et al., 2023). In a study tackling the treatment and advancement of artificial stem cells, mice were injected with stem cells modified or supplemented with angiogenic and/or neurotrophic genes and proteins. These artificial stem cells were targeted towards improving erectile organ function. Results showed a positive synergic effect between the stem cells and the modifications added,

confirming good efficacy in treating organ dysfunction (Argiolas et al., 2023). The use of combinatorial artificial stem cells as a means of early intervention for organ dysfunction reveals the potential use of SASCs across disease progression to address cellular senescence.

Stress-Induced Proliferation

Cell proliferation is the process by which cells grow and divide as per the cell cycle. Although both cell proliferation and differentiation concern cell development, it is crucial to differentiate the two terms. Proliferation is the increase in cell numbers, whereas differentiation is when specialized cell types are developed from unspecialized stem cells. In this review, proliferation is the main focus, as uncontrolled proliferation is a primary factor in tumor formation and the growth of cancerous cells. Stress-induced proliferation is the constant growth and division of cells, specifically due to stressors. While many stress factors underlie the causes of cell proliferation, this review focuses on oxidative stress, which is prevalent in inducing cancer cell proliferation. Excess reactive oxidative species (ROS), such as hydrogen peroxide and nitric oxide, lead to metabolic disturbances in cell growth, proliferation, and apoptosis. Therefore, oxidative stress-induced proliferation results from the presence of more reactive oxidative species than what antioxidants can regulate to maintain cell homeostasis. *In vitro* studies suggest CRISPR and SASC technologies can detect and target signaling pathways and stress response genes. To target reactive oxidative species overproduction, current CRISPR research focuses on enhancing the activation of oxidative stress response transcription factors (OSRts). Reactive oxidative species scavenging and antioxidant treatments through SASCs induce cell senescence to halt proliferation without causing damaging cell death.

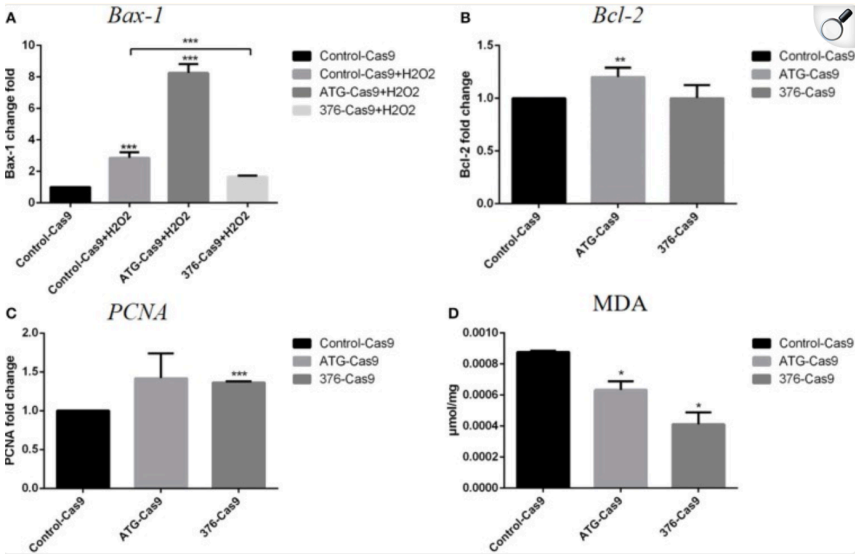
CRISPR Techniques for Stress-Induced Proliferation

CRISPR/Cas9 mechanisms have been used to intervene in gene pathways that lead to stress-induced proliferation. A key application of CRISPR in stem cell research is its ability to regulate differentiation. By precisely controlling gene expression, scientists can guide stem cells to develop into specific cell types, such as neurons, muscle cells, or pancreatic cells, which is essential for developing targeted cell therapies for conditions like Parkinson's disease, heart disease, and diabetes (Lino et.al, 2018). Moreover, CRISPR can be used for epigenetic modifications through gene activation (CRISPRa) or inhibition (CRISPRi) without permanently altering the DNA sequence. This capability allows researchers to study gene function and develop new therapeutic strategies without the risk of unintended genetic changes (Lino et.al, 2018).

CRISPR can increase antioxidants to control high levels of oxidants within the stem cell environment. The nuclear factor erythroid 2-related factor (Nrf2) is an OSRt that regulates gene detoxification and response to oxidative stress. Specifically, the *Keap1*-Nrf2 pathway is crucial to understanding oxidative stress response. The *Keap1* gene includes oxidative stress sensor cysteines and forms part of an E3 ubiquitin ligase that regulates Nrf2 activity (Baird & Yamamoto, 2020). Hu et al. (2020) utilized CRISPR/Cas9 to disrupt the *Keap1* gene, which releases, activates, and localizes Nrf2 into the nuclei of adipose-derived mesenchymal stem cells (Ad-MSCs). By editing the *Keap1* gene, the study found a lower content of malondialdehyde (MDA), which is a biomarker of membrane lipid peroxidation (Hu et al., 2020). Membrane lipid peroxidation occurs when cell membranes are damaged due to oxidative stress. Thus, the low MDA is associated with increased stress resilience with the help of Nrf2 released from the *Keap1* codon binding (see Figure 1).

Figure 1

CRISPR/Cas9-Induced Loss of Keap1 Enhances Anti-Oxidation in Rat Adipose-Derived Mesenchymal Stem Cells



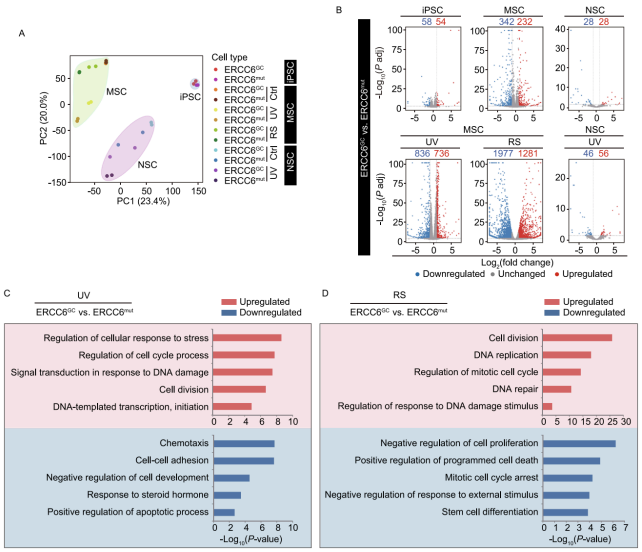
Another notable example is the p38 MAPK pathway, a stress-activated signaling cascade that drives stress-induced proliferation in hematopoietic stem cells (McGuire & Arthur, 2016). Dysregulated activation of the p38 MAPK pathway in hematopoietic stem cells (HSPCs) promotes increased levels of reactive oxygen species and triggers DNA damage response mechanisms (Della Volpe et al., 2024). While this response initially helps replenish lost blood cells by increasing proliferation, chronic activation of this pathway leads to stem cell exhaustion. This causes HSPCs to lose their long-term regenerative capacity. Additionally, genomic instability caused by excessive DNA damage increases the risk of an accumulation of genetic mutations, potentially leading to diseases like myelodysplastic syndromes or leukemia, where uncontrolled hematopoiesis results in disease progression.

Della Volpe et al. (2024) recently showed that during CRISPR-Cas9 editing of HSCs, activation of a p38 MAPK-ROS axis caused “proliferation stress” and DNA damage in those cells. In other words, the process of gene editing itself can induce a stress response. Importantly, the same study demonstrated that treating cells with a p38 inhibitor before gene editing significantly improved outcomes. Inhibition of p38 before CRISPR editing slowed the cell cycle and expanded the population of HSPCs, ultimately giving the edited cells increased differentiation capacity. This finding suggests that modulating stress pathways (like p38/reactive oxidative species signaling) can prevent the exhaustion of stem cells that might otherwise occur during gene editing procedures.

CRISPR has also been applied to correct genetic defects that make stem cells overly susceptible to stress. For instance, Wang et al. (2020) used CRISPR/Cas9 to repair a mutation in the *ERCC6* gene in induced pluripotent stem cells (iPSCs) derived from a Cockayne syndrome patient. Cockayne syndrome is a neurodegenerative syndrome marked by premature aging defects and increased sensitivity to oxidative stress. *ERCC6* is a gene that encodes a DNA repair factor involved in transcription-coupled nucleotide excision repair. This pathway is crucial in fixing DNA damage caused by oxidative stress, which Cockayne syndrome patients are highly sensitive to. After gene correction of *ERCC6*, the iPSC-derived mesenchymal stem cells showed a reversal of their stress-related defects: correcting the ERCC6 mutation using CRISPR/Cas9 proved effective in reversing the premature aging characteristics observed in the patient’s stem cells, restoring their resistance to DNA damage and normalizing their proliferation rates (see Figure 2). This example highlights how CRISPR-based gene editing can reinforce the genome’s ability to handle oxidative stress, thereby treating and preventing stress-induced proliferation.

Figure 2

Transcriptional differences between Cockayne syndrome (CS)-specific and gene-corrected (GC) cells across different conditions



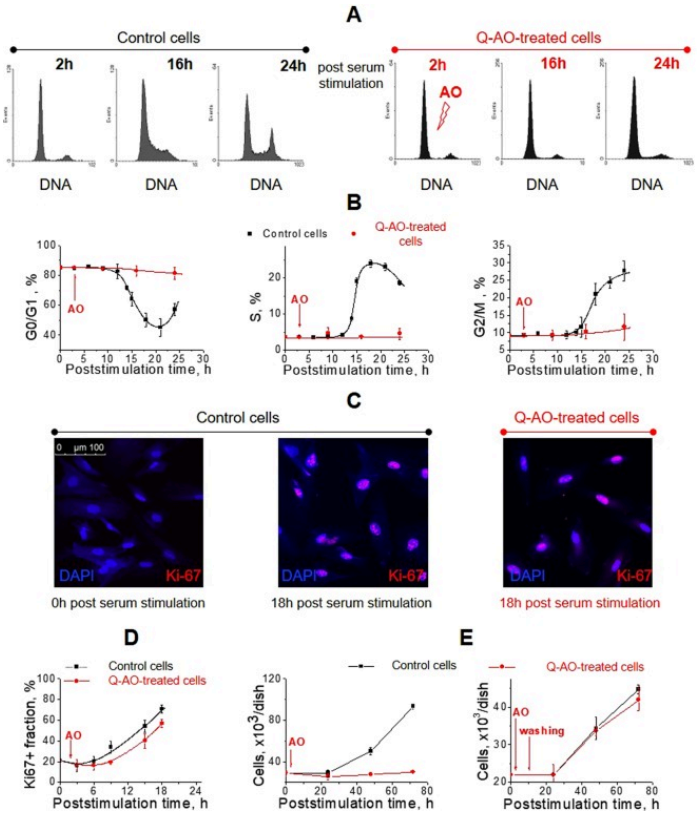
SASC Techniques for Stress-Induced Proliferation

Synthetic artificial stem cells are utilized to mimic natural stem cells for greater stem cell function and survival. While CRISPR focuses on releasing antioxidant response by manipulating signaling pathways, synthetic artificial stem cells are cultivated with antioxidants to control stress-induced proliferation. The antioxidant treatment on SASCs has been proven to target reactive oxidative species, thus blocking the initiation of the proliferating cycle. The effects of the non-cytotoxic antioxidant doses are found in Kornienko et al.'s study on quiescent versus proliferating mesenchymal stem cells. The research observed that high doses of the antioxidants induced reversible blocks of cell proliferation without any genomic consequences. However, when applied to proliferating cells, the same dose caused DNA damage and activation of the

pATM/pp53/p21 pathway (Kornienko et al., 2019). The study concluded that the dosage of antioxidants can result in both anti-aging and anti-proliferation benefits, along with further unwanted DNA damage in the cell. With the appropriate amount of dosage, antioxidant treatments on stem cells will be able to target both cell aging and proliferation efficiently (see Figure 3).

Figure 3

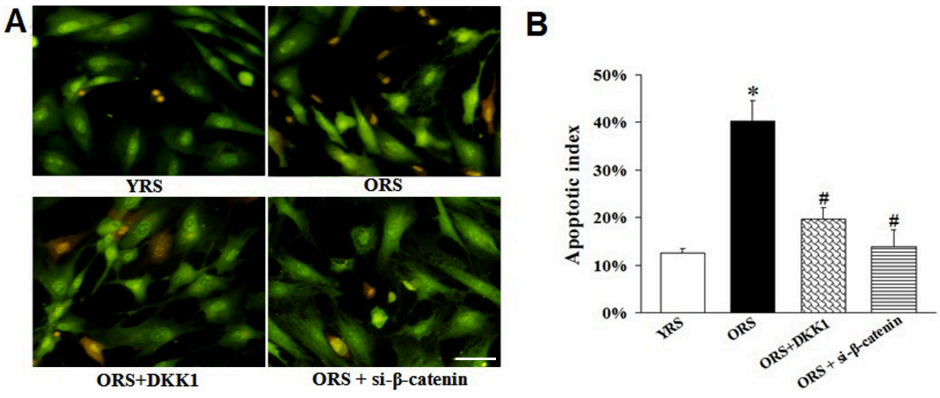
Antioxidant (AO) treatment of quiescent endometrial mesenchymal stem cells (eMSCs) leads to a G0/G1 phase cell cycle arrest. (A–B) Flow cytometry histograms and quantification show suppression of cell cycle progression after AO treatment. (C–D) Immunofluorescence imaging and Ki-67 analysis confirm G1-block. (E) Graphs reveal reversibility of the G1 arrest upon AO removal.



While antioxidant treatments block excess cell proliferation, microfluidic technology is used to manipulate synthetic stem cells with precise control to scavenge reactive oxidative species. Using the microfluidic electrospray technology, microcapsules with an enzyme-based reactive oxidative species elimination system were developed (Wang et al., 2023). The reactive oxidative species-eliminating pathway in the study contained enzymatic inverse opal hydrogel particles (IOHPs) that were capable of reactive oxidative species removal by multi-stage enzymatic catalysis. Immobilized superoxide dismutase (SOD) and catalase were encapsulated and successfully underwent a cascade reaction to detoxify the reactive oxidative species and maintain the redox balance within the cell (Wang et al., 2023). Reactive oxidative species scavenging and catalysis have been proven to reduce reactive oxidative species without the input of antioxidants or effects on cell aging and proliferation, providing an alternative means of engineering stem cell treatments (see Figure 4).

Figure 4

Wnt/β-catenin signaling induces the aging of mesenchymal stem cells through the DNA damage response and the p53/p21 pathway



Discussion

Cell senescence and stress-induced proliferation present two unintended consequences of dysfunctional cell cycle regulation. While both have physiological purposes in normal circumstances, they can easily lead to the formation of tumors or other degenerative diseases like cancers or other diseases arising from gene mutations. Throughout recent studies on stem cell treatments, CRISPR and synthetic stem cell (SASC) use has shown promising results in achieving long-term therapeutic results, in ways as much as replacing a patient's stem cell pool with healthy, unmutated cells. CRISPR has had relatively significant success in correcting genetic mutations, with a primary example being its ability to target the TERT gene and delay cell senescence by stimulating telomerase enzymes in repairing telomeres. The inhibition of the p38 MAPK pathway leads to delayed cell aging and thus the suppression of cell senescence. In addition, stress-induced proliferation from excess reactive oxidative species is another target that can be mediated through CRISPR and SASC technology. Activating antioxidant stress response systems and inputting antioxidants into the cell is the main focus toward mediating oxidative stress on cells.

As the examples mentioned above rely mainly on external manipulation of base genetic composition, this creates the opportunity for CRISPR/Cas9 technologies to be combined with the use of synthetic stem cells for a combined treatment. Given the current limitations of producing embryonic stem cells as well as ethical debates over its use, synthetic stem cells offer a viable solution that can still achieve the necessary treatment goals in correcting mutations or preemptively replenishing the stem cell pool and avoid physiological complications later on, as seen in the use of preemptive stem cell transplants. Between research on both cell senescence and stress-induced proliferation, Cockayne syndrome was a key focal point shared between both

areas of research, with general agreement on the potential of stem cells in correcting pathogenic mutations through external transplantation. Stem cell transplantation has been consistently shown to be effective in supplying cells that contain no pathogenic mutations and thus could replenish somatic cell counts, but data specifically indicate the necessity of the utilization of the CRISPR technique in editing the genetic components of these mesenchymal stem cells. In both instances, the age-related effects of Cockayne syndrome were reversed within the cells, and the patient exhibited effective recovery.

As the topic of this review implies, a combined treatment option will be most viable and development in creating synthetic artificial stem cells (SASCs) most similar to that of embryonic or maternal stem cells, particularly those originating from umbilical cord blood, to achieve maximum compatibility and effectiveness. As implied in the study by Sanberg et al., preventative stem cell transplantation during the primitive neonatal stage can ensure protection against neonatal as well as adult diseases. However, given the inconsistent nature of the availability in obtaining stem cells from the mother, SASCs present an alternative for stem cell banking and will still allow for the same improvement of cell aging in organs. While the study itself listed a possible application of maternal stem cells as reducing the need for artificial stem cells, allowing synthetic artificial stem cells to still be able to take on diverse genetic identities depending on the target organ and recipient can still be highly beneficial, especially in environments where prenatal treatments or collection of maternal stem cells are not an option.

The importance of preventing both cellular senescence and stress-induced proliferation is further highlighted by diseases like cancer. Although cell senescence is important in preventing cancer cells from abnormally proliferating, it can actually increase the aggressive quality of cancer by creating an immunosuppressive environment (Yang et. al, 2021). Furthermore, stress

hormones can increase the risk of DNA damage that promotes tumorigenesis, or the creation of cancerous tumors, and the unregulated proliferation of cancer cells. This stress-induced proliferation is key in tumor growth and metastasis (Pérez et. al, 2018). Similar functions for both of these cellular behaviors are present in many other diseases, especially those that are neurodegenerative or age-related. Finding a dual solution for both senescence and stress-induced proliferation in stem cells thus becomes vital in attempting to cure the root causes of such diseases. Since abnormalities in stem cells are possible root causes for many degenerative diseases, including rheumatoid arthritis and cancer, it is vital to target these functions in stem cells specifically to not only treat, but prevent, such diseases (Vignjević Petrinović et. al, 2023).

With the analysis conducted, cell senescence and stress-induced proliferation have been indicated to often arise from the same physiological and mutagenic causes, and a combined treatment involving both CRISPR and SASCs is shown to be one of the most viable and sustainable solutions. For example, a potential combination of CRISPR technology that edits both Keap1 and Mdm2 to activate Nrf2 and p53 may suggest a positive regulation of oxidative stress response, thus reducing abnormal cell proliferation. Antioxidative treatments in SASCs with the proper dosage will be able to introduce a combined treatment for cell aging and proliferation. Furthermore, both CRISPR and SASCs may be used to enhance reactive oxidative species scavenging and catalysis, which aids the redox balance of cells. Future research on this topic should delve deeper into the possibility or limitations of directly editing the DNA sequence of synthetic stem cells, as well as how their results compare with those of embryonic or maternal stem cells. Stem cell research presents its own set of ethical dilemmas, but by using the greatest possible use of synthetic artificial stem cells, stem cell treatments may become more accessible and ethically accepted in the future.

Conclusion

Cellular senescence and stress-induced proliferation represent two opposing yet interconnected challenges in regenerative medicine. While senescence functions as a protective mechanism against abnormal cell proliferation, it also presents barriers to tissue regeneration. Conversely, stress-induced proliferation, when uncontrolled, contributes to tumorigenesis and other pathological conditions, including fibrosis, neurodegenerative diseases, and chronic inflammatory disorders, where excessive cell growth disrupts normal tissue function. Addressing both processes requires innovative approaches, such as CRISPR-mediated gene modifications and synthetic artificial stem cell (SASC) technologies, which offer promising avenues for enhancing cell function and longevity.

CRISPR gene editing has been utilized to regulate telomerase activity and reverse senescence through Yamanaka factors, while SASCs have shown potential in modulating oxidative stress to prevent premature aging. Additionally, CRISPR techniques targeting stress-response pathways, such as Keap1-Nrf2 and p38 MAPK signaling, have demonstrated promise in mitigating oxidative damage and abnormal cell proliferation. These advancements highlight the convergence of gene therapy and stem cell research in developing targeted treatments for age-related degeneration and proliferative disorders.

Using gene editing technologies like CRISPR/Cas9 and synthetic artificial stem cell techniques for cellular senescence to strategically change genetic composition and expression within stem cells allows for profound treatments using the ability of stem cell differentiation. Synthetic artificial stem cells challenge cell aging by using combinatorial methods to supplement damaged cells. Additionally, CRISPR/Cas9 techniques for stress-induced proliferation develop therapeutic strategies without the risk of unintended genetic changes. Avenues that involve

correcting gene mutations using CRISPR/Cas9 techniques proved to be effective in reversing the premature aging of individual stem cells. CRISPR-based gene editing tools can reinforce genomic power to tackle stress and disease, aiding in treating and preventing stress-induced proliferation. With accurate and targeted manipulation, treatments on stem cells can address the issue of cell aging and proliferation efficiently.

CRISPR/Cas9 techniques hold a profound effect in the capability to correct genetic mutations to help repair telomere shortening, genetic diseases, and cell damage. Using CRISPR/Cas9 techniques, stem cell transplantation is effective in providing non-pathologically-mutated cells that could aid in restoring somatic cells. The differentiation of artificial stem cells into diverse genetic identities is a crucial benefit to treatments where the collection of parental stem cells is not a viable alternative. While current research is limited, the proposed methods for understanding advanced techniques for stem cell therapies provide an insightful avenue for future research.

Despite these advancements, current research remains largely confined to *in vitro* trials, with clinical applications requiring further validation. Future studies should focus on refining these technologies for *in vivo* applications, improving gene editing precision, and ensuring the safety of synthetic artificial stem cell therapies. With continued development, the integration of CRISPR and SASCs holds immense potential to revolutionize regenerative medicine, offering innovative solutions for aging-related diseases, cancer therapies, and tissue repair.

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Figures

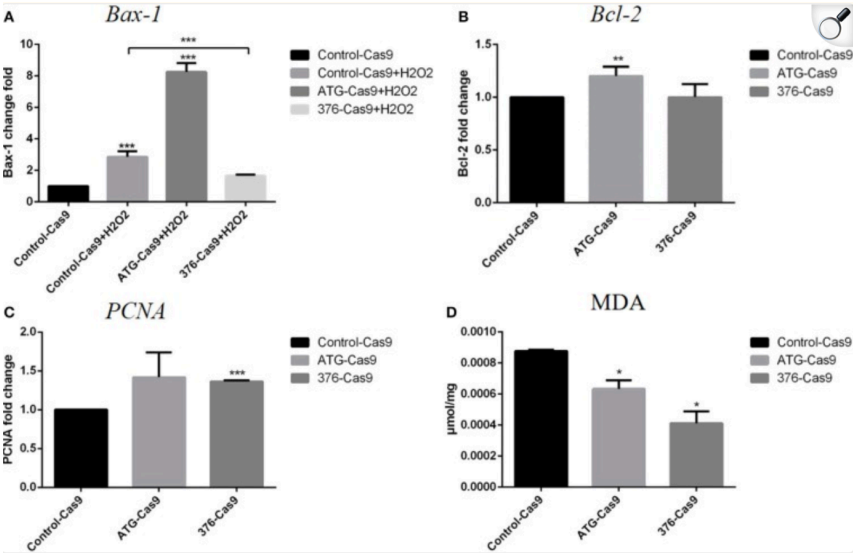

Figure 1: CRISPR/Cas9-Induced Loss of Keap1 Enhances Anti-Oxidation in Rat Adipose-Derived Mesenchymal Stem Cells

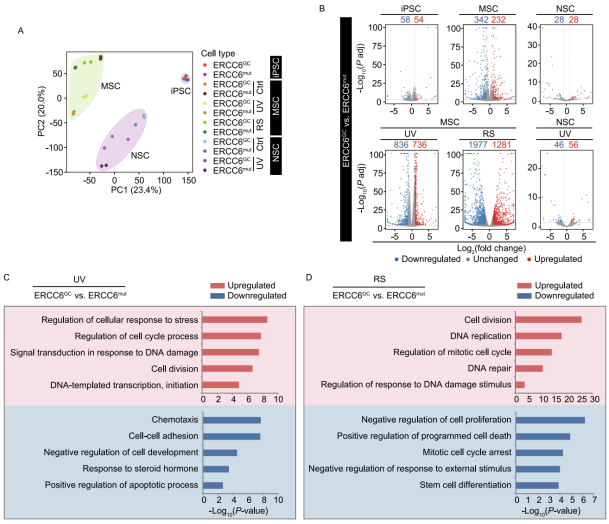

Figure 2: Transcriptional differences between Cockayne syndrome (CS)-specific and gene-corrected (GC) cells across different conditions

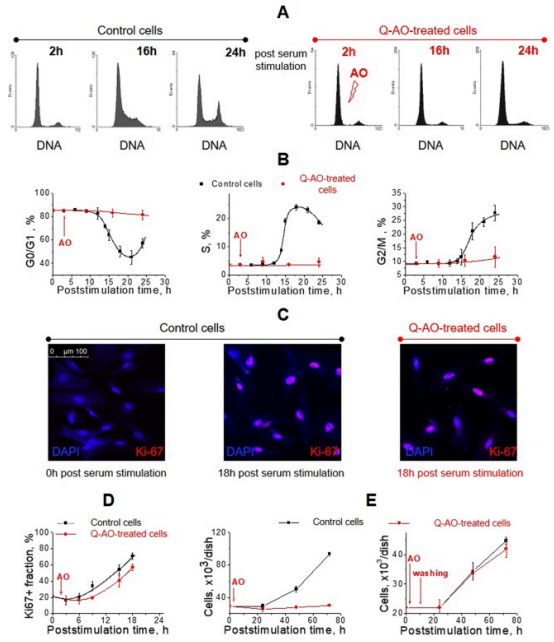

Figure 3: Antioxidant (AO) treatment of quiescent endometrial mesenchymal stem cells (eMSCs) leads to a G0/G1 phase cell cycle arrest. (A–B) Flow cytometry histograms and quantification show suppression of cell cycle progression after AO treatment. (C–D) Immunofluorescence imaging and Ki-67 analysis confirm G1-block. (E) Graphs reveal reversibility of the G1 arrest upon AO removal.

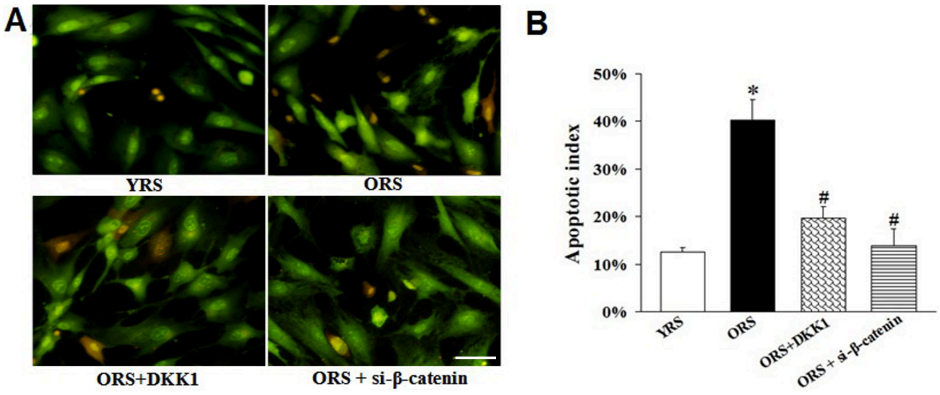

Figure 4: Wnt/β-catenin signaling induces the aging of mesenchymal stem cells through the DNA damage response and the p53/p21 pathway

Acknowledgements

We would like to thank the Undergraduate Laboratory at Berkeley (ULAB), Public Health and Health Sciences Division, for their mentorship and guidance throughout the research and writing process. We additionally want to acknowledge the University of California, Berkeley, for providing access to the scholarly works cited in this paper.