Rejuvenating the Elderly – A Scientific Approach for Tackling Geriatric Intensive Care

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Abstract

Continuous advances in science and technology have been slowly increasing the mean life expectancy on a global scale. It is expected that the number of individuals over the age of 80 will triple within the next three decades. In response to this, there is an increasing demand for additional space in geriatric intensive care units (ICUs) hospitalizing patients with debilitating or life-threatening conditions that become exacerbated with age. Current avenues of treatment against illnesses and injuries seen in elderly ICU patients have been predominantly based around prevention and improving quality of life. The few treatments that address the disease directly, if any, are rarely applied to geriatric patients due to the reported high mortality rates of procedures that are challenging to handle in an aged state. In this review, we highlight recent advances using induced pluripotent stem cells generated from patient-specific reprogrammed somatic cells as a possible route of non-invasive therapy for the treatment of conditions that normally warrant admission to geriatric ICUs. We also touch base with hurdles that have yet to be overcome before benchtop science can be translated for human use. Finally, we speculate about the possible future direction that personalized regenerative medicine using reprogrammed cells may take based on scientific research that is currently being carried out.

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Introduction

According to the World Health Organization, the year 2015 has marked the first time in recorded history where the number of people aged 65 or older outnumbered children under the age of five.[1] Indeed, due to increasing scientific and medical advancements in the healthcare field, the average life expectancy continues to rise from the age of 50 in the 1900's to the age of 81 in most present day developed countries.[1] However, improvements in longevity do not often coincide with an improved quality of life. The elderly population is one of the demographics at the highest risk for developing fall related injuries with nearly half of individuals aged 65 and over experiencing this at least once a year.[2] As one might expect, old age is considered the primary risk factor responsible for major debilitating and life-threatening conditions, including mental health and cardiovascular disease.[3] As a result, there is an increasing demand for geriatric intensive care units (ICUs) specializing in the management of infections and physical injuries. However, due to stringent admissions criteria and limited bedding space, it is estimated that only 40% of elderly patients (older than 80 years) are referred to the ICU, with only half of them gaining admittance.[4] Moreover, those that were discharged were found to have up to 60% to 80% mortality 12 months after ICU hospitalization.[5-7] Although the exact cause of this remains unclear, there is evidence to suggest that the aged state of the patient contributes to a poor outcome after a treatment or procedure.[8-10]

To date, most therapies for geriatric ICU patients are preventative in nature and directed at improving quality of life post-treatment. Although effective at providing short-term care after an injury or illness, this approach falls short in comparison to the strategies that must be developed for the future, where it is expected that 16% (1.5 billion) of individuals worldwide will be above the age of 65.[1] In order to address this, efforts are currently underway to rejuvenate the aged population. Herein we provide a thorough overview of recent advancements in stem cell biology and regenerative medicine directed at improving outcomes in geriatric patients.

The Discovery that the Age of a Cell can be Reversed

In 2006, Shinya Yamanaka discovered that cells could be reprogrammed into a state of pluripotency via the introduction of four transcription factors: Oct4, Sox2, c-Myc, and Klf4

(now called the Yamanaka factors).[11] This finding, for which he and the English developmental biologist, John Gurdon, were awarded the Nobel Prize for Physiology or Medicine in 2012, revolutionized the field of regenerative and personalized medicine. Shortly after this breakthrough, several research teams around the world were reporting that the conversion of mature somatic cells into an induced pluripotent state appeared to change the reprogrammed cell's epigenome to one normally seen in younger or developing cells.[12-16] It became clear that the reprogrammed cells underwent several changes while in a pluripotent state upon re-differentiation back into their original somatic identity. The most drastic and relevant of these changes were the elongation of telomeres (the caps on chromosomes that shorten with age), repair of reactive oxygen species-induced damage to DNA and mitochondria, and extensive chromatin remodelling.[17-20] Furthermore, when this epigenetic or nuclear reprogramming using defined factors was applied to senescent or terminally differentiated cells (i.e. metabolically active cells that have lost the ability to divide), there was an observed ablation of the cell's Hayflick limit and restoration of its proliferative potential.[21-23] In addition, rejuvenated cells were found to be more resilient to injury or damage and displayed improved function when compared to the unrejuvenated control group.[24-26] Although promising, the findings presented herein have been limited to simple or cell-based systems that are not always an accurate representation of what happens on the level of the organism. Nonetheless, these preliminary experiments are effective at proving the concept that a cell's biological age and function can indeed be rejuvenated under controlled conditions. The following section will describe what happens when the concept of rejuvenation is applied at the level of the organism.

Whole Body Rejuvenation in Animal Models

Regenerative and reparative capacity following injury decrease with age.[27] As a result, physical age remains a large factor for determining admittance into an ICU.[28] The problem lies not so much with the debilitating illness of the patient, but rather, the procedure that they must undergo in order to treat the condition. For example, many geriatric ICUs report poor outcomes in elderly patients following the administration of anesthetics prior to surgery, having to undergo cardiopulmonary resuscitation or requiring mechanical ventilation, when compared to younger individuals undergoing similar procedures.[29,30] Although efforts could be dedicated into formulating safer routes of treatment or advocating for lifestyle changes as a preventative measure, it is clear that these approaches delay rather than address these issues.

The past few years have seen a surge of scientific research directed at rejuvenating elderly individuals in order to enhance resilience against injury and improve outcomes after treatment. Arguably the most promising approach towards the revitalization of internal structures in aged patients is through the continuous introduction of stem cells obtained from a younger source.[31-33] We and others have shown that a young, but not old, source of allogeneic stem cells improves functional recovery after injury in aged mice.[34-38] Indeed, it is accepted that the stem cell niches in different organs become depleted with time, and this contributes to impaired turnover and differentiation of new cells that can then be used for tissue repair as well as maintaining homeostasis and optimal levels of organ function.[39-43] Moreover, targeted insertion of stem cells into specific regions has proven very challenging due to migration of these cells to different areas and very high apoptosis levels following transplantation.[44-46] To address these problems, we have developed chimeric mouse models using aged mice 18 months or older whose bone marrow (BM) have been reconstituted to comprise primarily of young (2-month-old) hematopoietic and mesenchymal stem cells. At baseline (in the absence of injury), we observed that these transplanted young stem cells, which were engineered to express green fluorescence protein, were seen to migrate out of the BM and embed themselves in many different organs including the heart, brain, lungs, liver, kidneys and so forth. Behaviorally, our chimeric mice displayed greater locomotive and exploratory initiative when compared to control animals which had their BM reconstituted with aged stem cells. Moreover, after inducing a myocardial infarction or cerebral ischemia, we noticed that these 24-month-old mice with a BM composed of young stem cells displayed significantly better ability to repair damaged tissue, higher resilience to injury, and improvements in function resembling levels seen at baseline before the injury when compared to controls.[37,47,48]

To date, the vast majority of rejuvenation research has been limited to *in vitro* cell and *in vivo* animal models. Although rodent models of rejuvenation have shown promising findings in terms of improving function and decreasing mortality after injury and treatment, whole body rejuvenation in humans has yet to be accomplished due to this field of research being a fairly new concept with insufficient basic science support.

Autogeneic Stem Cell Transplantation Using Rejuvenated Cells in Geriatric Patients

Shortly after the discovery of an inducible pluripotent state using defined factors, it was found that mature somatic cells reprogrammed by this manner reverted to a DNA methylation signature that is normally seen in cells from 6-month-old infants.[49] This finding opened up the possibility of using pluripotent stem cells derived from aged cells from elderly individuals, which are normally senescent and unable to further divide, as a source of differentiated cells for autologous cell replacement therapies. Since then, life-threatening and debilitating conditions that are commonly reported among the elderly in ICUs have either been treated or have had their severity attenuated using patient-derived induced pluripotent stem cells. An example of some of these conditions include Alzheimer's diease,[50-52] dementia,[53-55] heart failure,[56-58] and chronic obstructive pulmonary disease.[59,60] Moreover, positive outcomes after treatments and procedures were often improved when cell therapy was combined with gene therapy61.

In vivo Reprogramming Using Defined Factors

An alternative approach towards using patient-derived induced pluripotent stem cells, which normally requires a biopsy followed by *ex vivo* reprogramming and then finally engraftment of these cells into a specific targeted location, is

reprogramming diseased or impaired cells directly while they are still inside the body. The generation of various specific cell types from easily accessible patient cells, such as skin fibroblasts or BM stromal cells, ultimately still requires transplantation of these cells. This raises several problems such as the number of cells that engraft, which is dependent on how many cells become integrated and survive after transplantation, purification of these cells and finally producing enough of them to be able to meet a minimum level for an observable therapeutic effect.

In vivo reprogramming gained traction roughly 1 year after the Nobel Prize was awarded for the discovery of an inducible pluripotent state. Since then, personalized therapies have been developed that have been shown to improve quality of life and allow management of otherwise terminal and debilitating conditions seen in elderly ICU patients. Some of these conditions include inflammatory bowel diease,[62] spinal cord trauma,[63-65] ischemic stroke,[66-68] heart disease[69,70] and cancer.[71-76] Although promising, *in vivo* reprogramming of cells has many more layers of complexity involved than *ex vivo* reprogramming followed by transplantation. The primary concern is that *in vivo* reprogrammed cells have a high tendency to turn into cancerous cells, most notably teratomas, which are tumors composed of the three different germ layers.[77-81] As a result of this, many clinical trials that were using *in vivo* reprogramming of diseased cells have been put on hold or completely terminated.[82-86] Despite this setback, efforts are still underway to circumvent this and other barriers. A brief summary of the challenges that are commonly faced when using reprogrammed or rejuvenated cells, as well as where the next steps should be taken in this field of regenerative and personalized medicine, are outlined in the following section.

Challenges and Future Direction

Since their discovery, induced pluripotent stem cells have changed the way medicine diagnoses and treats diseases. However, this breakthrough using reprogrammed cell-based therapy does come with several drawbacks that make it extremely challenging to translate most benchtop science into clinical trials and bedside treatments.

There are three main barriers that must be overcome when working with induced pluripotent stem cells. The first is the low reprogramming efficiency of somatic cells into an induced pluripotent state. For every 1000 somatic cells, only 1-10 of them will become reprogrammed, and this is entirely dependent on whether a protein- or RNA-based reprogramming approach was used, in addition to other variables such as whether the factors were introduced virally or non-virally.[87-90] The second challenge lies in producing enough of these reprogrammed cells in order to be able to differentiate them into a sufficient number of somatic cells that can then be used for therapy. Inducing proliferation in rejuvenated cells, especially when they are terminally differentiated cell types, is very challenging. This is why c-Myc and Klf4 (the last two Yamanaka factors) are employed, which are oncogenes that are designed to enhance the proliferation of cells during and after the reprogramming phase.[91,92] The difficulty with the activation of these genes leads to the third problem with using induced pluripotent stem cells, and that is the high likelihood that these cells will become tumorigenic following c-Myc and Klf4 expression.[93] Even though these two factors can be replaced with Nanog and Lin28, respectively, doing so results in lower yield of cells as well as diminished reprogramming efficiency and does not completely ablate the probability of tumor formation both *in vitro* and after transplantation.[94-97]

Although rejuvenation by epigenetic reprogramming to pluripotency by defined factors and subsequent re-differentiation has only been around for a little over a decade, many of the challenges that arise during basic science and clinical trials are quickly being solved. Very recently, a group was able to safely reprogram cells *in vivo* in mice with high efficiency without any tumor formation using a doxycycline-inducible method of gene expression by introducing the reprogramming factors into the animals' drinking water.[98] Unfortunately, the researchers also observed a high mortality rate due to untargeted reprogramming of somatic tissues that were vital for maintaining the survival of the animal (i.e. heart and lung cells). Other groups have found that genetic abnormalities (such as those causing sickle cell disease), could be completely ablated when these cells were reprogrammed into an induced pluripotent state and then re-differentiated back into their original cell type.[99-104] Finally, a breakthrough was made half a decade ago demonstrating that highly-proliferative solid-state tumors could be epigenetically reprogrammed into a terminally differentiated lineage, such as a red blood cell, which lack a nucleus and therefore lack a means of division.[105]

It is clear that induced pluripotent stem cells have the potential to improve quality of life after injury or genetic-based diseases in individuals residing in ICUs. This is especially true when it comes down to elderly patients, who experience a high mortality when subjected to conventional therapies against illnesses that in the future can otherwise be treated using the patient's own skin cells. However promising, more basic science needs be done in order to address crucial problems that arise during and after the reprogramming process. Once these roadblocks are bypassed, it is clear that autogeneic stem cell therapies will be the future face of medicine. Treatments will be DNA-specific to every patient with no fear of rejection or tolerance build-up. Medication will be cellular rather than chemical in nature, leading to greater specificity of treatment with less side effects and improved positive long-term outcomes.

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