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Regenerative Medicine and its Potential in Cardiovascular Disease

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ABSTRACT

Regenerative medicine, using stem cell therapies, has shown great promise in developing advances in medicine to long standing disease. Gene editing technologies such as the novel CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats with CRISPR associated protein 9) system, has enabled gene manipulation that is precise and efficient, with applicability to regenerative medicine. Patients with diseases thought to be incurable may attain the most benefit from regenerative medicine, particularly with cell-based therapies. Induced pluripotent stem cells due to their versatility and flexible differentiation have proven that they are the most viable stem cell option in the creation of safe and effective regenerative treatment. To create induced pluripotent stem cells, somatic cells first need to code for an induced pluripotent stem cell in the body using varying levels of the following transcription factors: cMyc, Klf4, Sox2, and Oct4. Once in an induced state, these pluripotent stem cells need to be further specialized and differentiated into a specific cell. To code for this differentiation into a cardiomyocyte, additional transcriptional factors are required, including: Gata4, Mef2c, and Tbx5. Through the alteration of these seven different transcriptional factor levels, an ideal induced pluripotent stem cell and eventually the ideal cardiomyocyte can be created. With the development of functional cardiomyocytes, micro arteries can be created which can aid in redirecting blood flow away from blockages caused by diseases such as myocardial infarctions. This can greatly reduce the fatality of myocardial infarctions and prevent relapse.

Introduction

Picture a world in which a solution for long-standing diseases exists. Diseases thought to be incurable, like retinitis pigmentosa, can be cured by regenerative medicine (Akram Abbar). These diseases have been plaguing the world for as long as one can remember and no scientific approach has come close, until regenerative medicine. Regenerative medicine is a relatively novel field, with its beginnings traced to around the late twentieth and early twenty first centuries. It involves extracting a patient's cells, manipulating them to create specialized cells, and transplanting them back into the body to serve a healing/regenerative effect. This new approach can be applied to various areas of the body (somatic and germ-line) and can create new approaches to altering disease. Regenerative medicine has the unique ability to alter the fundamental mechanisms of disease, and thereby offer treatment options to patients where there is significant unmet medical need (Morrie Ruffin). It has an extensive reach in both research and clinical practice. While initially developed for oncology, it has the potential to make great changes in other fields such as the cardiovascular field (Surabhi Sharma). Regenerative medicine has the ability to cure multiple hereditary and nonhereditary diseases that were seen to be incurable. It can use CRISPR CAS-9 to alter the structure of the heart into a desirable form, can alter gene levels to create a desirable induced pluripotent stem cell, and can manipulate induced pluripotent stem cells into cardiomyocytes for structural support.

Stem Cells and Use in Regenerative Medicine

To comprehend exactly what regenerative medicine is, stem cells first need to be defined. Stem cells play a major role in regenerative medicine and are the basis behind these therapies. Stem cells are cells that can renew other cells in the body (somatic and germ-line) as well as dividing to form more stem cells. Stem cells are programmable cells which can be programmed to differentiate into various specialized cells such as skin, nerve, and liver cells. They produce these cells, like a factory, and replace cells from damaged areas in the body. Once stem cells are differentiated into specific cell types, they cannot transform back into stem cells. How does this tie into regenerative medicine? The whole technology behind regenerative medicine is the use of these stem cells. Regenerative medicine aims to manipulate these stem cells in order to control how exactly the body regenerates. This is a completely novel way of attempting to cure disease and has already proven to be successful in its approach. Take the case of a scientist in Japan who used regenerative medicine to cure retinitis pigmentosa. Retinitis pigmentosa, a disease in which an extra blood vessel is formed in the eye, eventually results in complete loss of vision (Akram Abbar). Regenerative medicine was able to intervene and restore the patient's vision by using induced pluripotent stem cells. "Regenerative medicine with IPSCs restored vision in the patient's eye rather quickly and helped remove the disease from the patient." (Akram Abbar). Scientists and researchers have learned that our own cells can be altered and sent back into the body to duplicate by the process of mitosis. Cells plagued by disease can be removed, altered so that they no longer are diseased in a laboratory, and sent back into the body as healthy cells where they can then replicate and spread which eventually rids the patient from disease. According to Richard T. Lee, a professor of regenerative medicine at Harvard University, "In just the past decade, we have learned that any cell type from any patient, including cells from a blood sample or skin biopsies, can potentially be reprogrammed into a stem cell, and that patient's stem cell can generate billions of new cells of a variety of differentiated cell types, including cardiomyocytes, endothelial cells and neurons." This research in cardiovascular regenerative medicine is important because the heart is an area in the body with relatively low regenerative capacities.

Myocardial Infarctions

Heart attacks (myocardial infarctions) have long plagued the heart. Heart attacks occur when an artery in the heart is either partially or completely blocked by plaque build-up. Heart disease continues to be one of the leading causes of death in the United States and myocardial infarctions are responsible for many of these fatalities. Each year, approximately 659,000 people die in the US because of cardiovascular disease; that is one in every four deaths (CDC). Relapses of myocardial infarctions are extremely deadly and are much more fatal than the initial event. When a myocardial infarction happens for the first time, an angioplasty is usually performed by inserting a catheter into the blocked area and opening it by inflating a balloon. At this point, the artery has been expanded and a stent is generally placed in the appropriate artery in order to maintain the arterial structure and prevent the artery from collapsing. These stents, however, do not come without their respective risks. Since stents are a foreign object in the body and the body wants to reject it and reseal the artery, stents in rare cases can in fact collapse and cause catastrophic effects. "In some cases, blood clots can form in the medicine-coated stents and cause a heart attack." (University of Michigan) Regenerative medicine can serve as a replacement for the stenting procedure. By manipulating the arterial structure cells after an angioplasty, new micro arteries can be created for maintaining the blood supply (Surabhi Sharma). By delivering stem cells to the appropriate location in the heart, new micro arteries can theoretically be created with no foreign bodies involved. This theory is in clinical development.

CRISPR Cas-9

Regenerative medicinal treatment can be optimized through the use of gene editing technologies. Gene editing involves targeting a specific gene or gene sequence in the body and removing or altering it. For example, if a gene that did not perform its normal function in the body was identified, it could be removed and replaced with a gene that was meticulously engineered by researchers. This increases the expression of healthy genes and reduces risk of developing different diseases such as cancer. Gene editing has been seen as a promising solution to cancer as the cancerous cells can simply be taken out of the body through the novel technology and be replaced with healthy cells. One of the more promising gene editing technologies is CRISPR-CAS9. CRISPR shows great promise to cure "diseases such as ß-thalassemia, hemophilia A, sickle cell disease, cystic fibrosis, Duchenne muscular dystrophy, and hereditary deafness" (Akram Abbar). CRISPR-CAS9 stands for Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated protein 9 (Medline). This name suggests exactly what its function is; the CRISPR protein 9 creates a short RNA sequence that is used to mock the process of DNA replication. It then uses the CAS9 enzyme to cut out the length of the matching DNA strand. Researchers then use a customized DNA sequence and replace the sequence that was removed (Medline).

Gene editing technology has already made numerous achievements in correcting genetic mutations, searching for genes to help with cancer immunotherapy, and solving problems in organ transplantation (Yuanyuan Xu). These same principles can be applied to regenerative medicine. Arterial structure cells can be removed and replaced with ones that are genetically engineered by researchers. These arterial cells can be coded to be expanded and more durable than existing arterial structure cells. Certain genes in cells in the artery can be replaced with stronger, more robust genes that help keep the artery open and prevent myocardial infarction relapses. Since IPSCs have been shown to work effectively with regenerative medicine and can be applied to treat cardiovascular disease. As mentioned earlier, an angioplasty keeps the arterial structure expanded, allowing cardiologists to insert a catheter through it. From here, instead of inflating the artery and inserting a stent, regenerative medicine can be used. Specialized cells, cardiomyocytes, can be developed and they can function as the self renewing cells in the expanded artery. Stents, as mentioned before, provide a risk of relapse which can simply be averted through the use of regenerative medicine. Regenerative medicine will manufacture an effective cardiomyocyte as well as deliver it to the proper location. Future clinical research into the manipulation of certain gene levels can create the ideal cardiomyocyte to maintain the proper blood flow and almost completely eliminate the risk of heart attack relapse.

Immune Response and Resulting Approach

However, in order to introduce regenerative medicine treatment into the body, cells need to be recognized and accepted by the body without immune response. This transport aspect of regenerative medicine has proven to be difficult to overcome. Until recently, the immunological response to regenerative medicine was relatively negative and the body would often reject the treatment and treat the cellular therapy as an invading foreign body. Even through the use of IPSCs derived from the patient themselves, the immune system still rejected the treatment. Regenerative medicine progress has "been hindered by major histocompatibility complex-human leukocyte antigen (HLA) genes, which pose a major barrier to cell- or tissue-based transplantation."(Mike Ainsley). In order to combat this and provide effective treatment, a team at the University of California: San Francisco led by Sonja Schrepher found that by attaching a specialized protein to the front of the transport vector, it "created a "do not eat me" signal against immune cells" (Jason Alvarez). The protein which they developed, called CD47, acts as a cell surface protein and helps avoid macrophages which are part of the immunological response. This discovery has proved to be crucial to future regenerative medicine developments as it provides the means of getting treatment into the body effectively by creating a "mask" around the vector.

Induced Pluripotent Stem Cells

Induced pluripotent stem cells are more effective in therapies than other types of stem cells because of their flexibility and ability to transform into other cells. Embryonic stem cells (ESC)- stem cells derived from the embryo of an organism- may be a viable alternative but they pose ethical and moral concerns. Although IPSCs and ESCs are both pluripotent stem cells, meaning that they can self-renew and be developed from somatic cells, IPSCs have been shown to be a more advantageous option. Since developing an embryonic stem cell involves the destruction of a human blastocyst, it is considered by some as destruction of human life. A blastocyst created *in vitro* is a human embryo fertilized in a laboratory. IPSC development, however, does not involve destruction of a blastocyst since its production process does not involve dealing with embryos. Since it deals with somatic cells, no blastocyst or other organism needs to be eliminated to produce it. Additionally, embryonic stem cells have shown less legitimate progress in curing diseases. Although they have been speculated to have great potential in curing Alzheimer's disease, cancer, and genetic disorders, embryonic stem cells have not legitimately achieved anything in those fields; it has been strictly speculative (Deborah White). IPSCs have been shown to be able to discover new drugs and treat life-threatening diseases (Sharif Moradi). Even though IPSCs are adult cells derived from the patient, they also face some difficulties in the body's immunological response. Since an induced pluripotent cell is developed from the patient's body, the patient's somatic cells can be examined. By manipulating the gene expression in somatic cells, an induced pluripotent cell can be created (Harvard Stem Cell Institute). This approach is novel and more effective than the preceding method which was only successful in translating about 0.001% of somatic cells into IPSCs (Harvard Stem Cell Institute). Genes need to be altered and as discussed earlier, through the use of CRISPR-CAS9, it is possible. Genes will be removed and new ones will be inserted in an attempt to vary the gene levels and expression in order to craft the ideal IPSC. To convert body (somatic) cells into induced pluripotent stem cells, research from the Harvard Stem Cell Institute can be referenced. This research initially discovered that four transcription factors code for this conversion between cell types. These factors were cMyc, Klf4, Sox2, and Oct4. By manipulating or controlling these gene levels in different cells, researchers are able to create an ideal IPSC. However, Sox2 and cMyc may induce cancer (Harvard Stem Cell Institute). Since these two genes were linked to cancer, research in the regenerative medicine field was limited due to its devastating potential effects. The Harvard stem cell team led by Kevin Eggan and Lee Rubin discovered that a chemical named RepSox can act in place of Sox2 and cMyc. This chemical eliminates the need for two different genes as well as the risk of cancer. Regenerative medicine advances have accelerated because of the now relatively risk-free nature of the manipulation of Oct4, Klf4, and RepSox. One of these advances involves the development of specialized cells such as cardiomyocytes which make up the heart's muscle. IPSCs have shown through trials that they "can be differentiated into all kinds of tissue in mice, including cardiovascular and hematopoietic lineages, sperm, cardiomyocytes, and retinal cells" (Akram Abbar).

While IPSCs are beneficial for general stem cell development, they can further transform into a cardiomyocyte. Cardiomyocytes are the cells that make up the heart's muscle, and ultimately its structure. For the regenerative approach to prevent heart attack relapses, IPSCs should not be targeted, but rather cardiomyocytes. In order to transform an IPSC into a cardiomyocyte, the IPSC needs to undergo another transformation. This time, the transformation is from an IPSC state to a cardiomyocyte state. Instead of using the genes/chemicals in Klf4, Oct4, and RepSox, the conversion is conducted by three factors: Gata4, Mef2c, and Tbx5 (BMC). While limited research has been done on human test subjects regarding the conversion of IPSCs to cardiomyocytes, experiments have been conducted on mice to determine the proper level of expression needed to be induced by the transcription factors. One such study, "reported the direct reprogramming of mouse fibroblasts into induced cardiomyocytes (iCMs) through the introduction of three factors: Gata4, Mef2c and Tbx5" (BMC Part of Spring Nature). The reason why it is crucial to code for cardiomyocytes instead of leaving it in its IPSC form is because cardiomyocytes are relatively risk free and free of disease. They eliminate secondary diseases

and other abnormalities. Cardiomyocytes that are coded from IPSCs in the body have great potential and are "free of the secondary abnormalities that result from end-stage disease, comorbidities, and prolonged pharmaceutical therapy." (BMC Part of Spring Nature).

Cardiomyocytes

While IPSCs offer great benefits, cardiomyocytes are a better fit to be used with cardiovascular regenerative medicine because of their unique ability to perform certain tasks like heart tissue engineering. "To date, the greatest success in maturation of PSC-derived cardiomyocytes has been with transplantation into the heart in animal models and the engineering of 3D heart tissues with electromechanical conditioning." (Karbassi). With this ability, researchers are able to create three dimensional models of heart tissue and essentially craft a customized artery. With this freedom, they can theoretically change the arterial structure in any way that they desire. From here, testing can be done to ensure the proper three dimensional model needed in order to keep optimal blood flow after an angioplasty. By doing so, a permanent solution is created which eliminates the need of a foreign body such as a stent after a heart procedure.

Conclusion

Regenerative medicine can not only serve as a means of myocardial infarction relapse prevention but also through gene and factor manipulation in stem cells, diseases such as Parkinson's, Alzheimer's, and even different types of cancers can be eradicated. Through gene editing technologies, the most effective being CRISPR-CAS9, harmful genes or genes plagued by disease can be extracted and replaced with healthy genes that replicate and spread through the desired location. Tying all of this together with the capacities and advantages of induced pluripotent stem cells gives regenerative medicine in the cardiovascular field remarkable potential. New micro arteries can be developed which brings unlimited potential for curing disease. Through the creation of these micro arteries, blood flow can theoretically be redirected and heart attacks may become an issue of the past. Coupled with the novel discoveries made daily, such as with the curing of the retinitis pigmentosa, stem cell regenerative medicine makes leaps and bounds daily. Regenerative medicine is the future of modern medicine and will replace outdated medical procedures for generations to come. Stenting after an angioplasty will become a procedure of the past after the widespread adoption of regenerative medicine and its capacities.

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