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Human Embryonic Stem Cell Research vs. Alternative Stem Cell Research: Is there a compromise?

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Science has approached research on human stem cells from many angles. Excitement exists over the possible treatments that may come from research on human embryonic stem cells, but there are concerns about the ethics of such research. After analyzing the different methods science has applied to human embryonic stem cell research, I will explore the underlying reasons that scientists, ethicists, and theologians still disagree on human embryonic stem cell use. To this end, I am going to analyze different research studies that have been conducted, the results they have produced, and any completed clinical trials with human subjects. For example, in the fall of 2014, a clinical trial in Japan was started to treat degenerative eye disease with induced pluripotent stem cells (Cryanoksi 2014). The subject of this trial was the first human treated with these cells. With the results of this trial and other clinical applications, I will compare recent studies with past studies. Using a representative set of scholarly articles and ethical critiques, I plan to examine the research, clinical trials, and medical practices that have been done with embryonic stem cells versus human adult cells and other stem cells. I will then survey the application of this research in medicine, examine efforts to compromise (specifically those involving induced pluripotent stem cell research), and analyze the progress that is being made medically and ethically.

Government policy plays a major role in the embryonic stem cell debate in terms of the funding given to human embryonic stem cell (hESC) research and which hESC lines may be used for research. Policies change depending on the presidential party in office, which party has the upper hand in Congress, and influences from private political groups. In 1973 when the Supreme Court legalized abortion as the outcome of the Roe vs. Wade court case, the United States government became concerned about the aborted fetuses and their use in research. This concern led the government to ban any federal funding for embryos and fetal tissue in 1974

(Biotechnology Timeline 2012). Once embryonic stem cells were derived in 1998, the debate over funded and authorized research on embryonic stem cell lines arose, and restrictions were made on these two issues for human embryonic stem cell research. One of the main advocates for hESC research remains the National Institutes of Health (NIH), which is one of the largest government medical research agencies in the world. In August of 2001, President George W. Bush allowed NIH funding on already existing human embryonic stem cell lines since their discovery, but no new hESC lines could be created (Monitoring Stem Cell Research 2004). While this showed progress for federal funding on medical research with hESCs, many still believed Bush's policies to be very limited. President Bush slightly modified his guidelines in 2005. In March 2009, President Obama revoked the embryonic stem cell policy guidelines put in place by Bush, with the purpose of expanding "NIH support for the exploration of human stem cell research, and in so doing to enhance the contribution of America's scientists to important new discoveries and new therapies for the benefit of humankind" (Obama 2009). This led me to investigate how the research done now has been funded and how the funding is distributed between the research for human embryonic stem cells, adult stem cells, and induced pluripotent stem cells.

Three Cell Lines

To investigate the use of human embryonic stem cells (hECSs) versus adult stem cells and induced pluripotent stem cells (iPSCs) in science and medicine, a clear understanding of the differences between the three stem cell lines is necessary. Stem cells offer great potential in healthcare in terms of regenerative medicine and cell-based therapies, which are treatments in which damaged cell tissue can be repaired by specific cells differentiated from induced stem cells (Stem Cell Basics 2015). Understanding the different qualities of these three stem cell lines

will aid in interpreting the healthcare advantages as well as ethical issues associated with each cell line.

Found in multicellular organisms, stem cells are undifferentiated, unspecialized cells that are capable of mitotically replicating themselves over long periods of time. This self-replication is referred to as proliferation, and can aid some organisms in cell replacement and repair. An example of this is the presence of stem cells in the deepest epidermal layer of our skin, the stratum basale. Basal stem cells continuously replicate, pushing new cells up towards the surface of our skin, renewing our skin as dead cells fall away from its surface. Stem cells are also unspecialized, which means the cell does not have structures and functions specific to a certain tissue. However, they are capable of differentiating into specialized cells. Cell differentiation is when an unspecialized cell develops into a cell that has specific structures and functions. This leads to capabilities of cell-based therapy to treat many diseases (Stem Cell Basics 2015).

One specific stem cell type is human embryonic stem cells, which are stem cells derived from human embryos in the preimplantation-stage. The preimplantation-stage exists before an embryo is implanted on the uterine wall (Stem Cell Basics 2015). In research, hECSs are obtained from in vitro fertilization of eggs, outside of the female's body. The embryos of this preimplantation-stage are called blastocysts, and it is from the inner cell mass of blastocysts that hECSs are precisely derived (Barad, et al., 2014). hECSs possess the ability to differentiate into any tissue cells of the human body, specifically cells of the embryonic germ layers capable of developing tissues in the body. This ability is defined as pluripotency. hECSs also possess the quality of self-renewal, remaining in a state of undifferentiation, and therefore are good cells to keep cultured in a lab because they remain capable of differentiation (Narsinh, et al., 2011). hECSs are suspected to serve as a very promising treatment method to many diseases, such as

diabetes, heart disease, muscular dystrophy, etc., due to their capabilities of differentiating into any specialized tissue types (Stem Cell Basics 2015).

Human adult stem cells (ASCs), also called somatic stem cells, are those not derived from human embryos or reproductive cells. Adult stem cells are undifferentiated cells derived from the body. The difference between adult stem cells and hECSs is that adult stem cells can typically only differentiate cell types of the tissue or organ from which they originated (Stem Cell Basics 2015). Adult stem cells play a role in maintenance and tissue repair. They have shown to be effective in transplantation therapies, one example being the transplantation of adult bone marrow stem cells, or hematopoietic stem cells. In the 1950s, bone marrow transplantation was performed between identical twins. In 1968, bone marrow transplantation therapy was done on non-twin siblings, and in 1972, the first bone marrow transplant successfully occurred between a donor and patient that were unrelated. This was a huge stride in medical treatment using adult stem cells (History of Transplantation 2016). One disadvantage to human adult stem cells is that there is a limited quantity of them in tissues, and outside of the body they do not replicate well. However, it has been discovered that they are capable of being reprogrammed to form other cell types, such as induced pluripotent stem cells (Stem Cell Basics 2015).

Induced pluripotent stem cells (iPSCs) are reprogrammed human adult cells, capable of differentiating into any tissue cell type. In 2006, Shinya Yamanaka genetically modified adult stem cells by the transfection of genes for four fibroblast-specific transcription factors, Oct3/4, Klf-4, Sox2, and c-Myc (Yamanaka and Takahashi 2006). Transfection is the transfer of genetic material and fibroblasts are the cells that make up connective tissue in the body. These genes genetically manipulated the adult cell, inducing pluripotency and differentiation capabilities that mimic those of hESCs. One advantage of iPSCs is that they avoid many of the ethical problems

hESCs face because producing iPSCs does not require the destruction of human embryos. Also, iPSCs can be autologous stem cells, meaning they are derived from the same body or individual, so they are not subject to immune rejection like hESCs may be (Barad, et al., 2014).

Ethical Issues

The use of human embryonic stem cells in research has been a hot topic of debate since 1998 when the first embryonic stem cells were isolated and grown from human embryos (Stem Cell Basics 2015). The debate peaked especially after President George W. Bush in 2001 permitted funding on embryonic stem cell research only on already existing stem cell lines (Monitoring Stem Cell Research 2004). Since then, political challenges and changes have been made to permit the restricted use of stem cells in research and medicine. Embryonic stem cell research has been reviewed and challenged or supported from biological, medical, ethical, and religious standpoints.

The main issue of the hESC debate is that a live human embryo must be destroyed or broken down into its separate components in order for hESCs to be obtained. However, hESCs are very promising for understanding how to better cure diseases due to their capabilities of differentiating into any cell type that can then form tissues and organs in the human body (Stem Cell Basics 2015). hESC research could lead to a groundbreaking understanding of how cancers and diseases develop, as well as creating cell-based therapies from generated ESCs that would replace damaged or detrimental tissue in the body (Stem Cell Basics 2015). While hESC research has the potential to treat and possibly cure several diseases, many people are still opposed to the fundamental issue of a “person”, the embryo, being killed for the purposes of research.

After President Bush's funding policy was put into effect in 2001 and followed by many counterarguments, the President's Council on Bioethics emphasized that the main concern surrounding almost every argument and counterargument is the moral status of the human embryo (Monitoring Stem Cell Research 2004). Does the human embryo qualify as a who, possessing personhood, or as a what, equating to just a mass of cells? Many opposing hESC research believe that the embryo is a person upon conception, or fertilization. Several religions believe that not only is the embryo considered to be a person at conception, but it also has obtained its own soul (Sandel 2015). This embryo must then have the same undeniable human rights as you or me, once it gains the status of a person. This status of personhood comes from the fertilized egg cell being able to self-direct, integrate, and function as a progressive unit in the early stages of existing as a human organism (Hurlbut 2005). One opposing argument states that only after implantation of the embryo into the uterine lining occurs, which is typically after fourteen days, can the embryo be considered a person. This develops the idea that the human life is not equal at every stage in its development; at each phase, the organism is a different entity of life until it reaches full human status (Sandel 2015). This claim is disputed by those against hESC research, who claim the human embryo displays continuity throughout its entire existence and "is a whole living member of the human species in the earliest stage of natural development… [that] will, by self-directed integral organic functioning, develop to the next more mature stage" (Hurlbut 2005). A human adult was once an embryo, so the claim is made that the moral value of the embryo must therefore be recognized.

Human embryonic stem cell research brings forth the issue of the human dignity and inviolability of the embryo, if it were to be deemed a "person". The lack of regard and destruction of a human life would be immoral and would demonstrate the insignificance of a

human life. Dr. William B. Hurlbut made the claim that "As we descend into an instrumental use of human life, we destroy the very reason for which we were undertaking our new therapies; we degrade the humanity we are trying to heal" (Hurlbut 2005). A counterargument to this follows that even if the embryo is not found to be a person yet, it is still recognized as an important, life-giving entity and should not be destroyed for the purpose of weightless or inappropriate activities (Sandel 2015). hESCs would be used positively for the purpose of biomedical advancements and treatments, aiming to help those suffering from diseases. Embryonic stem cells can differentiate into specific cell types, possibly allowing for the regeneration of cells and tissues that can treat diseases and aid in therapy for macular degeneration, spinal cord injury, stroke, heart disease, diabetes, osteoarthritis, etc. (Stem Cell Basics 2015). By denying the use of hESC research, we are disregarding the human life of diseased individuals who could possibly greatly benefit from this research. In contrast to this argument, bioethicist Daniel Callahan, who presented his testimony in front of The President's Council on Bioethics, brings forth the idea that while medical research is important for advancements in healthcare, government funding could be put towards many other things that could improve human life, such as current treatment for diseases and better living conditions. Callahan claims that money could be "spent on something else that would bring great benefits as well, whether on public health, education, job-creating research, or on other forms of scientific research." He was emphasizing that the moral imperative to conduct research "becomes 'too high' when it begins to encroach upon, or tempt one to put aside, other important values, obligations, and social needs" (Callahan 2003). In contrast, the President's Council on Bioethics points out the argument that it is the motivation of biomedical science to help those suffering with pain and sickness rather than to save those embryos that are frozen in a

'freezer,' and the "claims of human embryos cannot simply trump the claims of promising medical research" (Monitoring Stem Cell Research 2004).

Another ethical view supporting the use of hESC research is that after conception and during embryonic development, the human embryo does not have functional capabilities of a human being, such as consciousness, nervous system maturity, and use of the senses. This supports the claim that the embryo cannot yet have the moral status of personhood. The opposing view on this matter identifies that there is not a defined moment of functional capabilities where we claim that right then the embryo can be classified as a person. Functional capabilities develop over time. Also, the argument can be made that "if human worth is based on actual manifest functions, then does more of a particular function give an individual life a higher moral value?" (Hurlbut 2005). Would those at different functioning capabilities, such as youth, geriatrics, intellectually disabled, etc., be considered less morally valuable?

An issue of concern is the comparison between the natural, unassisted embryo losses that frequently occur upon attempted conception versus the amount of embryos lost or destroyed due to hESC research. During natural conception attempts, many eggs may become fertilized into embryos by sperm; however, the rate of these embryos failing to implant themselves on the uterine lining and developing into a fetus is fairly high and occurs naturally (Sandel 2015). Those opposing hESC research claim that a natural embryo death is weighted much differently than an intentional destruction of an embryo, causing death. Natural death of the embryo in the uterus cannot be controlled, while intentional killing of an embryo in a research lab can be. The counterargument to this claims that the weight of a potential life is not the same as a living life, and "the way we respond to the natural loss of embryos suggests that we do not regard this event as the moral or religious equivalent of the death of infants" (Sandel 2015). In regards to *in vitro*

fertilization (IVF), many excess embryos are discarded in fertility treatments once the best embryos are chosen for IVF. This can be claimed to be the same as the destruction of embryos for use in medical advances and treatment. The morality of both embryonic sacrifices should not be treated differently (Sandel 2015).

Federal policy and funding of hESC dictates what research can be done and how the research can be conducted, and for this reason the question of funding is one of the major ethical controversies surrounding stem cell research Those opposed to hESC research claim that the federal government needs to protect the rights of the human embryos and human life, restricting embryo destruction and collection, no matter what the research gain may be. Many also disapprove of federal funds going towards research that parts of the public deem unethical. Those supporting hESC research argue that it is the government's responsibility to support the medical research that may lead to the decrease of suffering and disease prevalence within the government's nation. The government would be doing harm to the nation by not supporting hESC research (Monitoring Stem Cell Research 2004).

Additional ethical issues include concern over access to expensive patient-specific therapies that are developed. This issue revolves "around the lack of equal access to treatment based on socioeconomic status and quality of healthcare (Brind'Amour 2009). There is a fear that if life-saving therapies are found, only those in upper socioeconomic classes will be able to afford them. As stated by Daniel Callahan, "it would seem unjust for money to be invested in research that would knowingly end in treatments or therapies that could not be afforded by government trying to cover all citizens or available only privately to those with the money to pay for them" (Callahan 2003). Also, a feminist ethical view points out the issue of putting an egg donor's health and safety as risk during the invasive extraction of the egg needed to be fertilized

to form the human embryo. This is mainly a concern with hESC research if the donor is donating strictly for research and not for personal reasons of *in vitro* fertilization. This ethical issue will be discussed further when introducing somatic cell nuclear transfer (SCNT) research.

In efforts to avoid the moral controversies that hESCs brings forth, science has been working towards methods to produce cells that can act as hESCs without the same ethical consequences. An alternative method to human embryonic stem cells in biomedical research is the use of induced pluripotent stem cells (iPSCs). iPSCs have shown to be a positive alternative for reducing the ethical problems of hESC use and for reducing the risk of immune rejection, a challenge that hESCs face. They also help to reduce the issue of unfair public access and affordability to stem cell based therapies due to iPSCs' easy obtainability and productivity (Bind'Amour 2009). However, iPSCs do bring with them their own ethical issues. One issue raised is if the induced form of the stem cell, which is only embryonic-like but has the same capabilities, will be legally protected in the same way that hESCs are? While iPSCs are a newer discovery, the ethics pertaining to it will continue to unfold.

iPSCs are exact genetic matches to the patient, eliminating rejection by the immune system. However, this quality brings forth the issue of reproductive cloning. There is a difference between cloning for therapeutic purposes and cloning to a produce a human life. If iPSCs are ultimately capable of generating an embryo that would have the same genetic makeup of the patient the cells were derived from, many more issues will arise. The capability of cloning may lead to a "slippery slope of dehumanizing practices, such as embryo farms, cloned babies, the use of fetuses for spare parts, and the commodification of human life," an argument pointed out by Michael J. Sandel, a member on the President's Council on Bioethics in 2004 (Sandel 2015). The commodification of human life is giving an economic value to any aspect of the human

body, as if it were a commercial item. There is the threat that embryos and fetuses will be produced solely to treat the diseases of other humans. A counterargument claims that this is a claim that should not be taken lightly, but that the government can create strict regulations on banning reproductive cloning, restricting commodification, and regulating the time period of embryonic development (Sandel 2015).

A positive aspect of iPSCs is that they can be generated from fibroblasts in the skin layers, so this is much less invasive for the donor compared to the extraction of eggs. Since samples are taken from the skin layer, there is a risk of infection of the incision. There is also a risk of worsened pre-existing disease symptoms when a skin biopsy is performed, such as fibrodysplasia ossificans progressive, a disorder of the connective tissue (Yamanaka 2010). However, Changsung Kim recently stated that iPSCs can be easily generated from "patient specific cell sources, such as skin fibroblasts, hair follicle cells, patient blood samples, and even urine containing small amounts of epithelial cells, eliminating much of the extraction complications (Kim 2014).

The goal of biomedicine now in terms of hESC, ASCs, and iPSC research is to make advancements towards treatments and cures for diseases, attempting to avoid the ethical controversies.

Different Techniques

Now that each cell type has been identified along with its ethical implications, the different techniques of deriving these cell types must be examined in order to determine if there is a common ethical issue or if different issues arise depending on the extraction and formation techniques. With technologies emerging and replacing the use of hESCs, the ethical issues go

beyond the status of the embryo. Ethical concerns now include whether creating iPSCs or other embryonic-like cells compromises the biological integrity of the human being, what determines the value of a cell, and even what it means to be a human being or person. Somatic cell nuclear transfer can become capable of creating human life from an induced embryo if the right environment can be made, which causes concerns of unethical medical practices. iPSCs may also be capable of this in the near future due to their characteristics and rapidly advancing research. Does a hESC have a higher importance or significance than an iPSC, even though both function almost equally and can potentially create the same result? I am going to identify the specific techniques of derivation of each cell line, including clinical examples. I will examine how successful each technique has been, recognize what ethical, scientific, or medical issues arise, identify the ethics of the clinical research, and determine how each technique relates to the initial ethical issues.

iPSCs

Induced pluripotent stem cells (iPSCs) are derived from differentiated human somatic cells by epigenetics, eliminating the destruction of a human embryo and avoiding changing the DNA sequence. Epigenetics is the method by which cells are manipulated to change gene expression without altering their DNA sequence. Previously, the use of skin fibroblasts to generate iPSCs was favored. However, vigorous research has been done since the discovery of iPSCs in 2006 that has lead to the discovery that mononuclear cells, or cells with only one nucleus, can be collected and generated into iPSCs from several sources on the body, including "skin biopsy, hair follicle progenitor, muscle, bone marrow/mesechymal stem cells, lymphocytes, and…viable epithelial cells from urinal track" (Kim 2014). More options for the source of the cells allows for easier extraction from the body. From these tissue samples, the

somatic cell is reprogrammed by the over-expression of transcription factor genes by methods such as viral vector expression, plasmid transfection, mRNA translation, or protein transduction (Narsinh, et al., 2011). The four transcription factor genes initially found by Yamanaka for successful reprogramming were Oct4, Klf4, Sox2, and c-Myc (Yamanaka and Takahashi 2006). Scientists Thomson and Yu soon after found that genes NANOG and Lin28 in conjunction with Yamanaka's method can increase reprogramming efficiency (Zhang 2013).

The use of iPSCs for disease modeling and tissue regeneration has excited researchers in the medical field, leading to research that suggests personalized medicine and patient specific cell-based therapy will soon be practiced regularly to treat patients. For example, in 2012, researcher Hansen Wang conducted neurological disease modeling with disease-specific iPSC lines. These iPSC lines were derived from tissues of patients with Rett syndrome, Fragile X syndrome, Down syndrome, Angelman syndrome, Prader-Willi syndrome, and Timothy syndrome (Wang and Doering 2012). Wang acknowledges limitations to iPSC modeling of neurogenetic disorders, such as determining "whether typical traits of neurogenetic disorders can be observed in the context of iPSC models," emphasizing that results from iPSC modeling must be used in conjunction with other research methods before any conclusions can be made (Wang and Doering 2012). However, the use of iPSC lines for disease modeling will help lead to better drug screening, drug development, and cell therapy. iPSC models give researchers an opportunity to observe living central nervous system cells that function in a similar manner as the cells working inside of the diseased patient (Wang and Doering 2012).

Similar research with iPSC disease modeling has been conducted to study many other medical conditions such as Alzheimer's disease, Parkinson's disease, heart diseases, and blood disorders (Kim 2014). Changsung Kim is predicting a near future medical breakthrough,

claiming that “now we can test novel therapeutic options with samples from patients without limit. We can even regenerate needed tissue such as patient matching blood, muscles, and neuron.” (Kim 2014). In September of 2014, only six months after Kim made this statement, the first human trial with iPSC treatment was conducted to treat age-related macular degeneration (AMD) of a 70 year old woman in Japan. Ophthalmologist Masayo Takahashi generated the treatment cells and eye specialist Yasuo Kurimoto led the procedure. Fibroblast cells from a sample of the woman’s skin were reprogrammed into iPSCs and then differentiated into retinal pigment epithelium (RPE) cells. These new RPE cells were grown into a sheet to replace damaged RPE cells that were a result of the patient’s AMD (Cyranoski 2014). While the results of this first trial are still being monitored for effectiveness and teratoma formation, the iPSC-derived RPE cell implantation appears to be a huge success and advancement in medical treatment (Garber 2015). However, some complications have arisen with the second trial to treat AMD with iPSCs that was to be conducted shortly after the first trial.

This first trial used “individualized autologous iPSCs transplants”, which are transplants generated from cells that were derived from the same patient. However, when the iPSCs were reprogrammed from the second patient’s skin for the second trial, mutations were discovered in the cells that prevented the trial from continuing (Garber 2015). This identifies one of the issues with iPSCs, which is iPSCs “often acquire mutations and epigenetic and chromosomal changes in culture,” (Garber 2015). To potentially avoid this issue and lead to a continuation of more AMD treatment trials, Takahashi identified a new approach of using allogenic cells, or cells not from the same patient’s body, to derive iPSCs for RPE generation. These allogenic cells would come from already existing banks of iPSCs that were derived from donations of blood tissues. This would increase genomic stability and increase efficiency of the procedure. Problems that do

come forth with the allogenic method include the risk of immune rejection by the patient's body and the treatment efficacy of only partially matched cells (Garber 2015).

The future of iPSCs is clearly very promising. The first trial with their use has shown to be safe and successful thus far. iPSC research is helping scientists make strides toward understanding diseases better, while avoiding ethical issues of embryo destruction and unsafe cell extraction from patients. As I have mentioned in previous sections, ethical problems still exist in terms of fears of reproductive cloning, embryo farms, and commodification of human body parts. A study conducted in 2012 in China demonstrated that iPSCs are fully capable of replacing embryonic stem cells. Genetically modified piglets were produced from iPSCs and nuclear transfer (NT) cloning, a method that will be explained in further detail subsequently. In the study, pig iPSCs (piPSCs) were used as "donor cells for reconstruction of NT embryos by traditional cloning and handmade cloning" (Fan, et al., 2013). In the first experiment conducted, 11,923 cloned embryos from six piPSCs lines and 1,585 cloned blastocysts from a different set of piPSCs failed to develop to term in surrogate mother pigs. It was reported that another study used 22,260 manipulated embryos from piPSC lines and failed to produce a living cloned pig (Fan, et al., 2013). However, in the second experiment of this study, the scientists made modifications to their production of the piPSCs, method of transcriptional activity, and treatment of constructed embryos. Through this process, they were ultimately able to produce four living cloned piglets. While the longest any cloned piglet lived was only 32 days, this was credited to the NT process that is known to cause random death. Overall, the experiment demonstrated that pigs can be successfully reproduced by using piPSCs as NT donors, which makes researchers believe that "in the future, this discovery may allow the generation of genetically modified pigs after gene targeting of the piPSCs…represent[ing] an efficient way to produce genetically

engineered pigs" (Fan, et al., 2013). This study adds to the fear that soon it may be hiPSCs that are being experimented with embryo cloning and genetically engineered babies. As demonstrated in this study, thousands of piPSCs were destroyed. If hiPSCs become capable of creating human life and if this form of human life is at the scientists' disposal, it will be hard to contend that iPSCs have avoided the ethical issues raised by embryonic stem cell work.

Concerns relevant to the majority of the research being conducted now on iPSCs include issues of informed consent, privacy, and the rights of tissue donors. iPSCs have an unknown and expansive potential in scientific and medical research. Progress has already been rapidly made since the discovery of iPSCs in 2006, and their full capabilities cannot even be understood yet. In 2014, researchers reported a study that obtained the attitudes of patients donating their tissues for iPSC derivation and research. The main concerns conveyed were about "privacy, immortalization of cell lines, commercialization of human tissues, and the creation of gametes" (Dasgupta, et al., 2014). A main concern with privacy was whether donors' personal information and name would be protected if it turned out that their cells were of great value and more information was needed about the donor. Some were also concerned about whether their insurance would be affected if certain genetic information was discovered in their tissue (Dasgupta, et al., 2014). Many donors brought up the case of Henrietta Lacks and how her tissue cells, known as the HeLa cell line, were used without her consent. If their own cells became immortalized, donors felt that they would not be able to control what their cells would be used for, no matter what consent forms they signed in the beginning. This issue then returns to the privacy concern and identifying the donor in the case that a researcher may want information about the donor or a change in consent down the road when discoveries are made with that donor's tissue.

If discoveries are made with the cell lines from the tissue of donors that could lead to new treatments, medicines, or technologies, a concern exists of "who should be compensated for providing tissues and how any resulting profits from the commercialization of research should be distributed" (Dasgupta, et al., 2014). While some believe that their biological material is a donation and that they should not receive a profit for the work that someone else did, others believe that the success of a researcher would not have been possible without the donor's tissue. This issue again returns to the controversial debate over the HeLa cell line; many researchers profited greatly from this cell line while Henrietta Lacks and her family were not given compensation and did not even know about the impact of her cells. While this would not be the case today, many are still concerned with where their cells will end up, who will be making a profit, and if the cell line from which the profit is made is being used in a way that upholds the donor's morals.

Another concern the donors expressed was whether their induced cell lines would be used to create gametes, or reproductive cells. Many disapproved of the idea that their biological material could one day create another human being that they would not know, or that it would be used for the purpose of cloning. However, one donor emphasized that if their induced cells were derived into a gamete and then used to create another organ in the body that could save the life of another human being, he or she would not be opposed to that. It is certain that in order to alleviate some of these concerns, strict regulations should be made upon the consent forms given to donors, including informed consent, privacy restrictions, and clarity of anticipated cell line uses. While the use of iPSCs does possess its own ethical issues, the problems with iPSCs seem to have more attainable solutions than those of hESCs.

Nuclear Transfer

Another alternative to the use of human embryos to obtain hESCs is the method of somatic cell nuclear transfer (SCNT) to derive human nuclear transfer embryonic stem cells (NT-ESCs). Just like the derivation of iPSCs, SCNT avoids the ethical dilemma of destroying a naturally-occurring human embryo derived from a female. However, an embryo is induced with this technique, causing some of the ethical issues of hESCs to remain. SCNT is the process of inserting the nucleus of a somatic cell, or a body cell from a human (such as a skin fibroblast), into an enucleated oocyte, or human egg cell with its nucleus removed. The oocytes used are mature, metaphase II-arrested egg cells that have "cytoplasmic factors…[that] have a unique ability to reset the identity of transplanted somatic cell nuclei to the embryonic state" (Tachibana, et al., 2013). Metaphase II is simply a step in the second phase of cell division, which in this case is stopped. The embryonic state of the newly formed NT-ESC allows the cell to potentially have the same pluripotent and regenerative qualities of both hESCs and iPSCs (Trounson and DeWitt 2013). Studies with SCNT to create NT-ESCs were put on the back burner when the hype of iPSC research occurred after Yamanaka's discovery of iPSCs. However, in recent years, NT-ESCs have become a focus for many researchers.

Shoukhrat Mitalipov and his team of researchers from the Division of Reproductive and Developmental Sciences at the Oregon National Primate Research Center demonstrated that a primate-modified SCNT method was successful in "reprogramming rhesus macaque adult skin fibroblasts into NT-ESCs" (Tachibana, et al., 2013). With this evidence, his research team went on to examine whether human metaphase-II arrested oocytes would have the reprogramming capabilities to derive usable NT-ESC lines. Mitalipov's results, published in 2013, demonstrated that the nucleus of infant skin fibroblasts (the donor somatic cell) were capable of being fused

into the human MII-arrested ooyctes by method of spindle removal and virus-based donor-cell fusion (Tachibana, et al., 2013). Spindle removal is the removal of thin fibers called spindles that form during cell division. Virus-based cell fusion is using the cellular envelope of virus HVJ-E, or "from [an] inactivated hemagglutinating virus of Japan" (Tachibana, et al., 2013). In other words, HVJ-E is an inactivated virus that can be used as a cell-fusing agent. As a result of Mitalipov's SCNT method, five stable blastocysts were derived, which in turn produced four stable NT-ESC lines that demonstrated pluripotency, no abnormalities, and few transcriptional differences (Tachibana, et al., 2013).

The SCNT method still has limitations and obstacles that need to be further researched, and the optimal conditions for the NT method, ooyctes used, and somatic cells used must be examined. However, this reprogramming discovery has excited many medical researchers who believe that this alternative method to derive hESCs will be more beneficial than the use of iPSCs. Natalie DeWitt and Alan Trounson, researchers at the California Institute for Regenerative Medicine, wrote a review about Mitalipov's discovery, claiming that "the generation of SCNT-ESC lines in this study shows it is feasible to generate cellular derivatives that may be more robust, genetically stable, and 'adult-like' due to the absence of somatic cell memory and without the introduction of genetic elements and oncogenes used to derive iPSCs" (Trounson and DeWitt 2013). Mitalipov supports this statement by also claiming that after more research, NT-ESCs might prove to be more advantageous than iPSCs since the former contain "mtDNA almost exclusively originating from the ooycte…[which] ensures that NT-ESCs acquire the potential to produce metabolically functional cells and tissues for cell therapies" (Tachibana, et al., 2013). mtDNA is mitochondrial DNA that codes for organelle functions and

metabolic activity within an oocyte, which is a female reproductive egg cell (Tachibana, et al., 2013).

The discovery of NT-ESCs in regenerative medicine and cell therapy does make a stride towards eliminating some of the ethical debate of hESCs. However, just as with iPSCs production, the derivation of NT-ESCs brings forth ethical problems. Many believe that since SCNT produces an embryo from a human egg, that embryo still has the potential to form a human being so therefore its destruction strictly for research is unethical. Two researchers from the University of California San Francisco Program in Medical Ethics make the claim that those "who object to SCNT believe that creating embryos with the intention of using them for research and destroying them in the process violates respect for nascent human life" (Lo and Parham 2009). This argument takes us back to the initial ethical issues involved with hESCs that are trying to be avoided. However, many counter this argument by claiming that the "pluripotent entities" or induced embryos are "biologically and ethically distinct from human embryos" (Lo and Parham 2009).

Another main issue is the extraction of human oocytes from female donors, which has raised concern about the safety of the donor (Trounson and DeWitt 2013). With the fast-paced research of SCNT and drive to make discoveries, there is an increased fear of females being exploited for egg donations. Research has demonstrated that when creating embryos by SCNT method, hundreds of oocytes are used; some result in cloned embryos and others fail to survive (Beeson and Lippman 2006). The rate of success as of 2014 was only about 1 in every 200 attempts to create viable NT-ESCs (Kuen 2014). The necessary amount of oocyte donations from females to consistently have beneficial research and progress would be very challenging to maintain. The derivation of oocytes from donors is claimed to be a painful, invasive, and risky

operation. It requires a high dosage of hormones and drugs that stimulate the production of eggs in the female body at a rate much higher than normal production (Beeson and Lippman 2006). Risks of this operation include: ovarian hyper-stimulation syndrome (OHSS), infections, infertility, excessive bleeding, ovarian cancer, renal failure, and surgical complications (Lo and Parham 2009, Kuen 2014, Beeson and Lippman 2016). OHSS has been one of the main concerns, since patients are at a high risk of developing the syndrome and it can have devastating effects and symptoms (Kuen 2014). It is a primary concern that all donors are given adequate information and informed consent about the procedure and all of the risks it entails prior to agreeing to donate. Mahendra Rao and Maureen Condic, researchers of neurosciences and regenerative medicine, put the point well: "because SCNT requires the use of donated oocytes, NT-ESCs would be subject to regulatory requirements for assessment of donor-associated risk, and oocyte donors would need to be screened for both genetic and transmissible disease" (Condic and Rao 2008).

Also, the same issue that arose with iPSC research presents itself with SCNT method: who gets compensation for donations and discoveries? In addition to these issues discussed previously, now there is the concern of whether women in poor economic situations or in debt from education will subject themselves to risky oocyte donations in order to gain payment in return from researchers desperate for oocytes (Lo and Parham 2009). This returns to the issue of commodification of human body parts or tissues. If SCNT research begins making more discoveries and requiring more oocyte donations, there is fear of unethical methods of obtaining oocytes. There are claims that regardless of any risks of donation, donations for SCNT research should not entail a payment for the eggs, and only compensation for medical expenses should be given (Kuen 2014). Critics of these claims state that because oocyte donation has so many risks,

it will be harder to find donors without giving them some sort of payment for their contribution. There are volunteer donors for other procedures that are permitted to be given payment, such as donors for liver biopsy and female donors of oocytes for infertility treatments (Lo and Parham 2009). Overall, the tissue donation and oocyte extraction for SCNT has far more ethical issues and risks than the simple tissue extraction for iPSCs.

Adult Stem Cells

Another alternative research approach to discovering cell-based regenerative therapies without the use of embryonic stem cells is the use of adult stem cells (ASCs). ASCs are undifferentiated somatic cells of the body that function in maintenance and tissue repair. ASCs display multipotency, which means they can derive more than one specialized cell of the body, but not all types of tissue cells of the body like pluripotent stem cells (i.e. hESCs or iPSCs). ASCs can be derived from many parts and tissues of the body, including bone marrow, umbilical cord blood, placental tissue, peripheral blood, and adipose (fat) tissue. Peripheral blood is the blood circulating throughout the entire body, containing red blood cells, white blood cells, and platelet cells. The different types of adult stem cells discovered include hematopoietic stem cells, mesenchymal stem cells, neural stem cells, epithelial stem cells, skin stem cells, and cardiac stem cells (Stem Cell Basics 2015). Hematopoietic stem cells are any stem cells that are blood forming, or that give rise to blood cells. They are derived from bone marrow. Epithelial stem cells are those found in the lining of the digestive system. The derivation of all of these ASCs does not require the destruction of an embryo or any use of germ line cells. Many favor adult stem cell research because it avoids the ethical concerns that hESC research faces. This method also does not create an induced embryo or embryo-like structure.

Mesenchymal stem cells (MSCs) are derived from connective tissue of organs in the human body. MSCs are believed to be the most multipotent of the adult stem cell types, and they have been demonstrated to be capable of differentiating into cartilage cells, muscle and tendon cells, fat cells, bone cells, and other cells found in skeletal tissue (Roura, et al., 2015, Kim, et al., 2015). While these cells are only multipotent, the tissue cells that they are capable of differentiating into could potentially offer great stem cell therapies for those specialized parts of the body. Many studies have been conducted demonstrating that MSCs have an especially promising potential as therapy for cardiovascular disease. A group of researchers from Connecticut claim that "a wide variety of cytokines, chemokines, and growth factors are produced by MSCs, and many are involved in restoring cardiac function or regenerating myocardial tissue" (Kim, et al., 2015). MSCs are patient-derived cells, providing safety against immune rejection. MSCs are also immune-privileged, meaning they can tolerate attacks from the immune system. This further allows these cells to be used in an allogeneic manner, or in patients with a different genetic makeup than from where the MSCs were derived (Kim, et al., 2015). This is a major advantage for potential MSC therapies. It was also demonstrated in research that MSCs can have enhanced therapeutic functions when they are pretreated with growth hormones (Kim, et al., 2015).

Many research studies have been done to demonstrate the best tissue source for MSC derivation. Of the studies done, umbilical cord, bone marrow, placental and adipose tissue have all been argued to offer the most usable MSCs for positive research results. Each study claims a different tissue source to be the best, demonstrating that research with deriving MSCs still needs to be further developed and many more trials need to be completed to accurately identify if one tissue is better than the other. The use of bone marrow for regenerative medicine and patient

therapies has been common since the 1960s. One study claims that bone marrow demonstrates the "strongest evidence for potential stem-cell based therapies" (Kim, et al., 2015). However, many recent studies claim that umbilical cord blood (UCB) provides the largest amount of stem cells for clinical application (Roura, et al., 2015). Umbilical cord blood has experimentally been shown to be more advantageous due to its ability to be "safely and painlessly extracted and long-term cryopreserved and has a lower risk of transmitting viral infections or somatic mutations than adult tissues (i.e. bone marrow)" (Roura, et al., 2015). This was especially demonstrated in cardiovascular research. Mesenchymal cells derived from UCB possess low immunogenicity and the capability to be used for allogeneic transplantation (Roura, et al., 2015). A human clinical trial in Korea used UCB-MSCs transplantation on four patients to successfully improve the patients' Buerger's disease and chronic limb ischemia. Buerger's disease is when obstruction occurs in the arteries and veins in the hands and feet, and the patients chosen for the clinical trial had already been treated with existing medical therapies that were unsuccessful. The results of the trial indicated that due to successful UCB-MSCs transplantation, all four patients had improved blood circulation throughout their limbs, shortened healing time for ischemic ulcers, and a reduction in pain (Kim, et al., 2006).

Other studies suggest that when adipose tissue is compared to bone marrow and placental tissue, "harvest and isolation of MSCs from adipose tissue consistently showed higher yields than MSCs" (Vangsness, et al., 2015). However, adipose tissue does require a more extensive processing after harvest than other tissue sources, which is unfavorable in a research setting. In 2014, MSCs derived from adipose tissue (AD MSCs) were used in a human clinical trial for articular cartilage regeneration and the treatment of osteoarthritis. The AD MSCs were injected into the knee of three groups of patients, each group receiving a different cell dosage. Results of

the clinical trial of patients in the high dose group demonstrated that the "osteoarthritic knee improved function and pain of the knee joint without causing adverse effects, and reduced cartilage defects by regeneration of hyaline-like articular cartilage," suggesting that with more extensive research and clinical trials, the use of AD MSCs for articular cartilage regeneration and treatment has much potential (Jo, et al., 2014). Overall, the promise of MSCs, regardless of which tissue they are derived from, appears to be great for stem cell therapies. As of 2015, there are currently 502 clinical trials with the use of MSCs in humans (Vangsness, et al., 2015).

While the possible therapies with ASCs are promising and already ASCs provide treatments for many specific diseases, they lose their appeal to many researchers due to their lack of pluripotency. So far, ASCs are limited to creating cell types of their same origin. ASCs also do not proliferate as well as hESCs once removed from the body, meaning they cannot undergo cell division and multiplication. This makes these cells harder to maintain in culture and in large supply for research (Hollowell 2002). A positive find in research that may help with the proliferation is the state of quiescence. ACSs are in a quiescence state in the body. Quiescence is when the cell cycle is arrested, preventing proliferation and differentiation of ASCs. This frozen state is capable of being reversed when the ASCs want to enter back into the cell cycle, becoming capable of proliferation again and therefore capable of being regenerative (Rumman, et al., 2015). Quiescence may be essential with future adult stem cell therapy research and helping the cells maintain their functions (Rumman, et al., 2015).

As with the use of iPSCs and NT-ESCs for future stem cell therapies, more successful research and clinical trials must be conducted for ASCs and more specifically MSCs to be considered an applicable and accessible treatment. Each technique presents its own limitations and/or ethical issues that must be addressed in each research setting and medical treatment

approach, because, as seen with hESCs, ethical complications can limit research capabilities and support. Even with the removal of the restrictions placed on hESC research by President Bush in 2009, there have been a small number of publications involving hESC research compared to alternative stem cell research (Esteso and Gearhart 2011). Much of the existing research has been done with mouse ESCs, and these studies demonstrate promise for future therapies that can be applied to humans. However, they too have to be further investigated and supported.

Several human clinical trials with hESC-derived cells have been conducted and deemed successful. The first attempted clinical trial was done by a company called Geron that used hESC-derived cells to treat spinal cord injury. This study was also the first clinical trial with hESCs to be approved for by the Food and Drug Administration (FDA). However, this study was stopped due to money and "regulatory issues" (Ratcliffe, et al., 2013). Geron continued to monitor the results of the study, but at the same time, Geron was conducting two phase-II cancer therapy trials and did not have the funds to complete their hESC study. Funds were reallocated towards these studies, placing the company's focus on oncology therapeutics (Ratcliffe, et al., 2013). In 2011, scientists of a company called Advanced Cell Technology, which is currently named Ocata, performed the first human clinical trial with hESC transplantation therapy that demonstrated positive clinical benefits (Ilic, et al., 2015, Schwartz, et al., 2012). The clinical trial used hESC-derived retinal pigment epithelium (RPE) to treat "patients with Stargardt's macular dystrophy and dry age-related macular degeneration" (Schwartz, et al., 2012). The study demonstrated the safety of hESCs since the following conditions were not present in any patient in the first four months: "hyperproliferation, abnormal growth, or immune mediated transplant rejection" (Schwartz, et al., 2012). The transplantation of hESC-derived RPE improved vision for patients with both diseases.

hESC clinical trials are currently being conducted, but the results are preliminary and most studies are focusing first on the safety of transplanting hESC-derived cells into humans. However, the issue of not only the safety of the patient but also the safety of the human embryo remains a main concern for opponents to hESC research. With these clinical trials, the hESCs used were derived from an embryo that was destroyed in the process.

Federal Funding

Those opposed to hESC research remain concerned over federal funds being used for research they find unethical. President Obama's 2009 Executive Order 13505 states that "the Secretary of Health and Human Services, through the Director of NIH, may support and conduct responsible, scientifically worthy human stem cell research, including human embryonic stem cell research, to the extent permitted by law" (NIH Guidelines 2009). This removed President Bush's restrictions on hESC research, creating controversy with hESC research opponents. In 2015, the NIH allocated a total of $1.416 billion towards stem cell research. Records from the NIH RePORT (Research Portfolio Online Reporting Tools) state the following funding support from NIH institutions or centers (NIH IC) to universities, laboratories, or medical centers: $180 million for hESC research, $159 million for non-human ESC research, $445 million for non-embryonic stem cell research, and $632 million for non-embryonic non-human stem cell research (NIH RePORT 2016). The RePORT shows data from 2012 to 2015, demonstrating that funding has decreased over the past four years in all stem cell research areas besides hESC research, which increased by $34 million since 2012. The 2016 estimated funding support by the NIH shows an increase in funds in all areas of stem cell research, ranging from $9 million to $26 million. An increase of $26 million is estimated for non-embryonic non-human stem cell

research, while an increase of $20 million is expected for non-embryonic human stem cell research, or adult stem cells (NIH RePORT 2016).

It is interesting to note that despite efforts by opponents of hESC research to block federal support for this work,, funding has increased and is expected to increase for the future. It is also important to include that the RePORT groups iPSC research in the same category as hESC research, allocating $180 million in 2015 to both kinds of research. This demonstrates that iPSC research is not getting the same attention as adult stem cell research and other forms of stem cell research stated above.

Conclusions

Science has discovered alternative methods for stem cell therapy research. The discussion is whether these alternative methods are sufficient and possess the same potential for patient therapies as hESCs possess, without the same ethical controversies pertaining to hESC research. Based on the above review, some conclusions can be drawn about the ethical problems presented and the most promising and least controversial stem cell research technique.

Overall, the moral status of the human embryo remains at the core of the ethical issues involved with stem cell research. This same issue arises with iPSC research, in that there is fear of their capabilities of creating induced-embryos. This fundamental issue also comes forth with SCNT, since this technique creates an embryo. The concern of the embryo remains at the forefront. iPSCs and NT-ESCs are considered to have the most promising comparison to hESCs, and yet they still encounter the main ethical issue. In order for iPSCs to surpass this ethical dilemma, strong governmental regulations must be made to prohibit unethical production of embryos. NT-ESCs can only surpass this issue if the created embryo can be deemed to have

lesser of a moral status since it was not created by the natural fertilization of a human egg. This matter, however, is of moral opinion and not scientific evidence.

Based on the findings for each technique and type of stem cell, it appears that iPSCs provide the most promising and genuine evidence of regenerative therapy with stem cells. iPSC research avoids the controversial use of an embryo, induces the least amount of harm or risk for the tissue donor, and provides positive results of clinical benefits. iPSCs surpass ASCs due to their pluripotency. They also surpass NT-ESCs due to ethical concerns of embryo status and risks of patient donation. With this in mind, I believe the NIH should be allocating more funds towards iPSC research. Based on the records of the NIH RePORT, iPSCs are not given the same support as other stem cell research. If compromises are to be made between the ethical dilemma of the destruction of a human embryo versus potential life saving stem cell therapies, the focus of federal funding and research should turn towards the most promising stem cell research.

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