The Pluripotency Proposition

A Biological and Ethical Case for the Utilization of hiPSCs in Place of hESCs

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A Senior Thesis submitted in partial fulfillment
of the requirements for graduation
in the Honors Program
Liberty University
Spring 2013

Acceptance of Senior Honors Thesis

This Senior Honors Thesis is accepted in partial fulfillment of the requirements for graduation from the Honors Program of Liberty University.

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Abstract

Human embryonic stem cell (hESC) research has spurred ethical controversy ever since it became feasible in 1998. The reason for this is due to the fact that hESC research requires the destruction of a human embryo, thereby causing the cessation of life for that developing human. Despite this unavoidable consequence, many advocates of hESC research hold to the belief that the embryo is not actually a human person, and therefore deem the destruction of the embryo as justifiable. Many advocates of hESC research also have pointed to the unprecedented medical potential of hESCs to argue in favor of their case. However, advocates of hESC research needlessly defend their position. This is because a new type of human stem cell with the same type of potential as hESCs was created in 2007. These new stem cells are referred to as human induced pluripotent stem cells (hiPSCs). hiPSCs are generated without the destruction of a human embryo, and thus avoid the ethical controversy associated with hESCs. Besides their ethical supremacy, hiPSCs have a biological advantage over hESCs due to their lack of immunogenicity that stems from their autological nature. This makes hiPSCs better suited for medicinal use in disease modeling, drug testing, and cell mediated therapy. These ethical and biological advantages are the reasons why hESC research should cease and hiPSCs should be utilized in their place.

History of the Stem Cell Debate

Biological History of ESC Research

Historically speaking, ESC research is at its infancy. Over the last sixty years the careful observation and problem solving skills of a few skilled researchers created the ability to generate a viable hESC line. In order to have a more complete understanding of the controversy surrounding hESC research, one must understand the biological history of successful ESC capture and cell line creation. This was an incredible accomplishment, and an understanding of the efforts that went into creating a viable hESC line will shed light on why many believe hESC research is not only optional, but why hESC research is necessary.

Teratoma and embyronal carcinoma research. Long before ESCs were collected from mouse or human embryos, research was being conducted on a unique tumor known as a teratoma. Histologically, a teratoma is recognized as a tumor containing all three of the germ cell layers, namely the endoderm, ectoderm, and mesoderm (1). These particular tumors have captured the interest of biologists and clinicians for years due to their tissue differentiation process that resembles early embryonic development (2). Teratomas served as the foundation for the discovery of ESCs. In 1954, Stevens & Little first discovered teratomas while examining mice testicles (2). Although most teratomas are benign, further research demonstrated that some of these teratomas were malignant and possessed the ability to be re-transplanted to consecutive hosts with subsequent teratoma proliferation. These malignant, re-transplantable teratomas are referred to as teratocarcinomas (2).

Teratocarcinomas contain a relatively undifferentiated tissue type referred to as an

embryonal carcinoma (EC). ECs had long been supposed to be the stem cell progenitor of the teratocarcinoma, and findings by Kleinsmith & Pierce in 1964 revealed that ECs did in fact have stem cell-like character (2). Murine EC cell lines were established in the 1970s in order to study this character and the phenomenon of tissue differentiation. Additional studies were being conducted during this time on the topic of teratocarcinoma origin. In 1970, research involving the implantation of teratocarcinomas to ectopic murine embryos revealed that teratocarcinomas have a germ line origin (3). The discovery of the germ line origin of ECs, along with the differentiation potential of ECs, led researchers to believe that ECs were similar to embryonic cells that possessed the same characteristics.

Search for the EC-ESC connection. The findings of the 1970s demonstrated that murine ECs possessed many characteristics typical of what was known about the embryonic inner cell mass (ICM) (2). These similarities had biologists desperately seeking to discover a connection between the two. Many biologists held that ECs were the malignant equivalent to those embryonic cells. In order to study this relationship, multiple research groups in the mid-1970s implanted EC cells into the uteruses of pregnant mice. Earlier studies had revealed that EC implantation on ectopic sites had resulted in teratoma formation (2). However, intrauterine implantation showed that these ECs became normalized in the presence of a normally developing blastocyst. These ECs also participated in embryonic development and differentiation (2). Furthermore, the daughter EC cells that participated in embryonic differentiation possessed a greater capacity for differentiation than their parent ECs in a number of studies (2). This was thought to be caused by an introduction of normal genes from the somatic cell. These

findings on the interrelatedness of ECs and ICMs gave rise to the collection and proliferation of ESCs.

Murine ESC line established. The link between ECs and embryonic cells was discovered in 1981 by two different studies led by Evans & Kaufman in one and Martin in the other (4) (5). Both studies documented the successful isolation of pluripotent cells from mouse blastocysts (3). These pluripotent cells, which came to be known as ESCs, were able to proliferate indefinitely and create cell lines similar to those of ECs when maintained in tissue culture (2). Although cells of the ICM do not normally proliferate indefinitely, ESCs are thought to do so because of their removal from the embryo. Additionally, when primordial germ cells are cultured *in vitro*, they develop into embryonic germ cells, which closely resemble EC cells and ESCs (2). These findings revealed that ESCs were the long sought after connection between ECs and the ICM. The successful capture and creation of a cell line of murine ESCs would not be isolated to mice but lead to the successful capture and creation of a cell line of human ESCs.

hESC line established. The successful isolation of hESCs did not immediately follow the successful isolation of murine ESCs. Human teratocarcinomas were first discovered in the 1950s, and human teratocarcinoma lines were established in the 1970s (2). After further research, several different human EC lines capable of differentiation were established in the early to mid 1990s. Although this served as a precursor to the isolation of hESCs, this accomplishment remained elusive due to biological, legal, and ethical factors (2). The human ECs shared some commonalities with murine ECs but had many distinct differences. These differences, coupled with the lack of ESC lines and direct information from human embryos, made it difficult to establish the connection

between humans ECs and ESCs that had been made between murine ECs and ESCs (2). After continued research, Bongso et al. were able to grow human ICM from a blastocyst of a donated embryo in 1993, and they were able to successfully isolate hESCs from the ICM in 1994 (6) (7). These were significant accomplishments, but the creation of a successful hESC cell line still eluded scientists. In 1995, Thomson et al. developed an ESC line from the rhesus monkey, and in 1996 Thomson et al. developed an ESC line from the marmoset (8) (9). Being of a primate origin, these ESCs more closely resembled the human ECs than the murine ECs or ESCs. Eventually, Thomson et al. were able to successfully isolate and establish a cell line of human ESCs in 1998 (10). Since this time an abundance of research has been published on hESC differentiation mechanisms, transplantation medicine, and disease modeling (11). Although the generation of a viable hESC line was a major scientific accomplishment, many individuals had reservations about this controversial topic.

Political History of ESC Research

The aim of hESC research is a noble one: To treat illnesses, cure diseases, and promote life. The controversy with this research arises over the means by which hESCs are collected. To date, the only methodology to collect hESCs involves the destruction of the human embryo (12). Although the ethical arguments both in favor and against hESC research will be examined more thoroughly later in this work, the ethical debate centers around this fundamental question: Is a human embryo a human person? Many who would answer “yes” to this question hold that the destruction of a human embryo should be equated with murder. 25 years prior to the first successful hESC line generation, this fundamental question had been examined by the U.S. government.

Abortion's effect on hESC research. The political history of the hESC debate cannot be divorced from the political history of the abortion debate. Since the *Roe v. Wade* decision in 1973, the issue of human embryo (and consequently hESC) testing has been highly politicized (13). Although the Supreme Court has not specifically ruled on the constitutionality of hESC research, this case has revealed where the courts stand on the personhood of a fetus, which presumably carries over to the personhood of the embryo (14). In this case the Supreme Court ruled that under specific conditions a woman is able to have an abortion on the grounds of a right to privacy for the mother (14). Implicitly, the Supreme Court determined that a human embryo has no inherent rights.

The importance of federal funding. Ever since the ruling on *Roe v. Wade* in 1973, the government has taken action to regulate the research of living embryos (13). Most of the political history of the hESC debate has centered on the subject of federal funding, and for good reason. Since hESC research is at its beginning stages, many private investors forgo investing in hESC research because they are unlikely to see any return on their investment within their lifetime (14). Thus, in order to move forward, hESC research is largely dependent on the government, but the federal government has been hesitant to comply due to ethical controversy.

The moratorium placed. In 1974 Congress placed a temporary moratorium on federal funding for clinical research on embryos and embryonic tissue until national guidelines could be established, although basic non-therapeutic research continued (13). The Ethical Advisory Board (EAB) was established in 1975 to establish protocols for *in vitro* fertilization (IVF). This protocol was established in 1979, but the Department of

Health and Human Services (DHHS) rejected these recommendations (13). EAB funding expired in 1980, and the Reagan and Bush administrations never extended funding. Therefore no IVF or embryo research was federally funded (14). In the late 1980s, the NIH attempted to gather federal funding for embryonic research, but the DHHS declined this request and the moratorium on embryo research continued (13).

The moratorium terminated. In one of his first acts as President, Bill Clinton lifted the ban on federal funding for embryo research in 1993 (13). The NIH Human Embryo Research Panel developed guidelines in 1994 for instances in which human embryo research should be federally funded. These recommendations included some instances where the creation of embryos solely for research would be justified (14). Congress, after becoming largely Republican in 1994, elected to ban federal funding for the creation of human embryos for research or for destructive embryo research. This ban was known as the Dickey-Wicker ban (14). However, Congress did not specify whether this applied to cells that had already been obtained from an embryo, so research on these cells continued (13). These political events set the stage for the arrival of viable hESC lines.

Dickey-Wicker and hESCs. When Thomson et al. had successfully created the first viable hESC line in 1998, the debate surrounding the Dickey-Wicker ban heated. Did hESC research constitute as destructive embryo research? The DHHS General Counsel, Harriet Raab, believed that since hESCs were not autonomous organisms, they were not embryos within the meaning of Dickey-Wicker (14). Several others disagreed. After the hESC line had been created, the NIH worked to develop guidelines for funding hESC research. By 2000 they had proposed that researchers could receive federal funding

for hESC research but only on hESCs that had already been derived from the blastocysts (13). In other words, the NIH would not fund the creation of new hESC lines from embryos. The NIH proposition hinged on a decision by new President George W. Bush.

George W. Bush's decisions. On August 9, 2001, Bush announced that he would allow NIH funding for hESC research, but only on hESC lines created prior to his announcement. No federal funding would be appropriated for the creation of new hESC lines (14). Advocates and opponents of hESC research were not completely satisfied with Bush's decision. Opponents disliked that hESC research was allowed to continue. Advocates disliked that their research was limited to previously established hESC lines, which possessed little availability, slight genetic diversity, and risked contamination and infection (14). Private hESC line creation could still continue though, and states such as California gave money for the creation of hESC lines (13) (14). A bipartisan bill to lift the federal ban on the creation of new hESC lines passed both houses of Congress in 2005, but President Bush vetoed this bill. The bill was reenacted in 2006, but it was vetoed once more (14). It was clear that President Bush was resolute in his decision, and more freedom for hESC research would not be given under this President.

Hope for hESC research advocates. hESC research advocates were pleased to see Barak Obama elected as President in 2008. In March 2009, Obama ordered that the moratorium on federal funding for the creation of new hESC lines be revoked (14). He did not, however remove all hESC research regulations. No current federal funding is given for the destruction of human embryos or for research with lines created solely for research purposes (14). New lines can only be created from leftover embryos since 2001 and those leftover from IVF procedures (14). Even with these lasting regulations,

Obama's decision has allowed for more freedom for hESC research and has delighted hESC research advocates. Nevertheless, there are many that still oppose hESC research, and the history of the hESC debate attests to the mixed opinions that individuals possess on this topic. This question still remains: Why are scientists so interested in researching hESCs? This nature of stem cells will give an answer as to why this is so.

The Nature of Stem Cells

Characteristics of Stem Cells

There are many different sources and types of stem cells, but each have two main distinctive properties. These are indefinite self-renewal and the capability to differentiate into multiple cell lineages, which is known as plasticity (15). Other cellular types may possess these characteristics, but it is the combination of these properties within stem cells that make them unique (15).

Indefinite self-renewal. Stem cells undergo cell divisions in order to self-renew themselves. Stem cells are able to do this either asymmetrically or symmetrically depending on the need of the organism (16). At the earliest stages of human development where there is little differentiation, stem cells undergo symmetric mitosis, meaning that both daughter cells retain their parent stem cell characteristics. This can also occur postnatally when the stem cell pool in certain tissues has been greatly depleted (16). Asymmetrical stem cell mitosis is when the parent cell mitoses into two intrinsically different daughter cells: one identical daughter cell and one more differentiated progenitor daughter cell (17). In this process, the parent stem cell polarizes itself by locating the cell-fate determinant molecules on one side of the cell. The mitotic spindle then aligns itself perpendicular to the cell axis polarity, and two different daughter cells

result after cytokinesis (17). Asymmetrical stem cell mitosis occurs in most postnatal circumstances in order to maintain the stem cell pool and to allow for normal tissue replenishment (16). Stem cells are able to renew themselves and proliferate indefinitely if they are in the correct environment. This ability is one of the links found between stem cells and the embryoid carcinomas discussed previously. It also poses one of the difficulties associated with stem cell research, which will be discussed later.

Plasticity. The second distinguishing characteristic of stem cells, their ability to differentiate into multiple cell lineages, is often termed plasticity. It is this characteristic that excites stem cell researchers because stem cells have the ability to become many different types of somatic tissues. This may have important implications for future medical practice. The level to which stem cells are able to differentiate is termed potency, which will be examined in the next section. In demonstrating plasticity, stem cells are able to cross lineage walls and adopt the phenotypic characteristics of cells of different morphologies (17). The plasticity of stem cells can occur by fusion or direct or indirect transdifferentiation. In fusion, a stem cell fuses with a cell of another type and can then express genes and display phenotypic characteristics typical of that cell (17). Direct transdifferentiation occurs when a stem cell acquires the identity of another cell by expression the gene pattern of that tissue. When a more differentiated cell reverts back to a state of less differentiation and then differentiates into a different cell type, that cell is said to have undergone indirect transdifferentiation (17).

Classification of Stem Cells

Potency classification. Stem cells may be classified in two different ways: by potency and by source. The potency of a stem cell refers to its differentiation potential, or

the amount of different tissues it is able to differentiate into.

Totipotent stem cells. The type of stem cell with the greatest differentiation potential is termed totipotent. A totipotent stem cell has the ability to successively divide and produce all differentiated tissues within an organism including any extraembryonic tissue, such as the placenta, umbilical cord, amniotic sac, and extraembryonic tissues that support these structures (18). The only cells that fall within this classification are the zygote up until the eight cell stage of the morula (17). Research also suggests that this totipotent quality is carried within the oocyte in the germ cells (18).

Pluripotent stem cells. The stem cells with the next highest level of differentiation potential are termed pluripotent. Pluripotent stem cells are able to differentiate and produce all three of the germ layer tissue types found within the ICM: endoderm, ectoderm, and mesoderm (18). Since pluripotent stem cells are able to generate all three germ layer tissue types, they are thereby able to differentiate into all adult tissue types. The major types of stem cells discussed in this work, ESCs and iPSCs, both belong under the banner of pluripotent stem cells. The fact that these cells can differentiate into any adult tissue is what gives them such promise in the field of medicine and makes them unique from other types of accessible stem cells being used in research.

Multipotent stem cells. Multipotent stem cells (MSCs) demonstrate the next greatest differentiation potential. MSCs are partially specialized cell types that are limited in the types of cells they can differentiate into (19). Hematopoietic stem cells (HSCs) are examples of MSCs. HSCs are able to differentiate into different blood cells but not into other types of tissue (19).

Unipotent stem cells. Lastly, the type of stem cell with the least differentiation potential is termed unipotent. These stem cells are only able to differentiate into one specific cell lineage. Unipotent stem cells are found in different body tissues where they act as stem cell reservoirs in the event that that particular tissue volume has depleted and needs to be renewed (19). Epidermal stem cells residing in the stratum basale serve as an example of unipotent stem cells.

Source classification. Stem cells may also be classified based upon the source from which they originate. The main sources for stem cells include adult stem cells (ASCs), umbilical cord stem cells (UCSCs), fetal stem cells (FSCs), ESCs, and iPSCs.

Adult stem cells. Adult stem cells are partially committed stem cells that are located within specific tissues and are able to convert and differentiate into the type of tissue in which they are located (19) Most ASCs are unipotent stem cells, but some are MSCs depending on the environmental factors of their tissue location (17). In their respective locations, ASCs serve as reservoirs capable of replacing damaged tissue (19). The microenvironment, along with physical contact and chemical communication among stem cells, stromal cells, and matrix, induce ASCs to differentiate and renew themselves (17). Mesenchymal stem cells are an important and frequently researched type of ASC. Although they are from a mesodermal origin, they are able to transdifferentiate into some cells of ectodermal and endodermal origins (19). Mesenchymal stem cells have also demonstrated the ability to differentiate in vivo and participate in bone tissue repair, immune system reconstruction, and revascularization of ischemic cardiac tissue in vivo (17). ASCs can be found throughout the body including in mesodermal tissues (bone marrow, muscle, adipose, synovium, and periosteum), endodermal tissues

(gastrointestinal tract), and ectodermal tissues (skin, deciduous teeth, and nerves) (17) (19).

Umbilical cord stem cells. UCSCs come from two different sources: umbilical cord epithelium (UCE) and umbilical cord blood (UCB). UCE has been shown to be an important source for human primary keratinocytes and is able to recreate the epidermis (17). UCB is a source of both hematopoietic and mesenchymal stem cell types. The umbilical versions of these stem cell types have demonstrated more tolerance than their adult counterpart (17). The umbilical cord mesenchymal stem cells can also produce cytokines which facilitate grafting in the donor (17).

Fetal stem cells. These are perhaps the least researched of all stem cell types. FSCs are MSCs and hold the same properties as ASCs (17). The source of FSCs for the sake of feasibility and fetal safety is fetal blood. A procedure known as ultrasound guided accession to fetal circulation is the method to obtain FSCs (17). FSCs are divided into five categories: hematopoietic, mesenchymal, endothelial, epithelial, and neural (17).

Embryonic stem cells. As noted previously, ESCs are pluripotent stem cells taken from the ICM of a developing embryo, thereby killing the embryo in the process. Their capture methods will be outlined in greater detail later in this work. ESC therapeutic potential and biological and ethical concerns will also be examined in a later section.

Induced pluripotent stem cells. iPSCs are somatic cells that have been reverted back to a state of pluripotency via the introduction of multiple transcription factors. Their derivation methodology will also be examined more intently later within this thesis. iPSC therapeutic potential and concerns with iPSCs will be examined later as well.

Pluripotent Stem Cell Derivation

Now that a biological and political context has been laid and the nature of stem cells has been discussed, the stem cells at the focus of this paper, hESCs and hiPSCs, will be examined in greater detail. Both of these stem cell types are pluripotent, making them highly desirable for research because of their great medical potential. Although they are both pluripotent, hESCs and hiPSCs are derived in significantly different manners and possess different qualities.

hESC Derivation

ICM collection. The first successful creation of a hESC cell line was reported in 1998 by Thomson and his affiliates (10). They used fresh and frozen human embryos that had been created by in vitro fertilization (IVF) for clinical purposes (10). IVF involves fertilizing a haploid ovum with a haploid sperm in order to form a diploid zygote. This zygote then undergoes several mitotic divisions until it reaches the blastocyst stage of early embryogenesis (12). The blastocyst contains an inner layer of cells called the embryoblast and an outer layer of cells called the trophoblast (12). The outer cell mass, known as the trophectoderm, forms the extraembryonic tissue that gives rise to the placenta, chorion, and umbilical cord (12). The embryoblast, or ICM, develops into the embryo (12). The cells of the ICM are isolated for the creation of a hESC line. In Thomson's study, he and his team cultured human embryos to the blastocyst stage and then isolated 14 ICMs (10). In Thomson's study and in the establishment of other early hESC lines, the immunosurgical method was utilized to retrieve the ICM (12). This method entails using anti-human serum antibodies and guinea pig complement to separate the ICM. However, the immunosurgical method was soon after discarded due to

its possibility of transferring pathogens and evoking an immune response in any implantation procedures (12). Currently mechanical dissection, enzymatic isolation, or laser beam isolation are used in the retrieval of the ICM from the blastocyst (12).

hESC characterization studies. Thomson tested his newly created hESC line to validate its credibility. These cells maintained their pluripotency, were karyotypically normal when grown on mouse embryonic fibroblast (MEF) feeders, and were able to form teratomas when grafted to severe combined immunodeficient (SCID) mice (12). Hence, these hESCs possessed the criteria of indefinite self-renewal and plasticity that are unique to stem cells (10). To date hundreds of hESC lines have been created, and all of these lines have resulted in the destruction of the human embryo (12). Although research has been conducted to develop a method of hESC derivation without embryo destruction, researchers have been unable to do so.

iPSC Derivation

Induction factor discovery. The first iPSCs were created from mouse embryonic fibroblasts in 2006 by Takahashi and Yamanaka (20). Tests were conducted on 24 candidate genes of transcription factors that were thought to be involved with inducing and maintaining pluripotency in ESCs (20). These genes were first individually introduced into MEFs by retroviral transduction, but no colony formation was noted (20). When all 24 genes had been retrovirally transduced, 22 different colonies had formed. Five of these possessed similar ESC morphology including a round shape, large nucleoli, and minor cytoplasm (20). Different combinations of gene transduction into the MEFs were attempted in order to determine which combination of factors was necessary to form a viable cell line that could be maintained in culture. After several trials, the combination

of four different genes proved to create viable pluripotent stem cell lines, and these factors caused greater ESC-like colony growth than the combination of all 24 genes (20). Those four genes were Oct3/4, Klf4, Sox2, and c-Myc (20). Thus, the combination of these four transcription factors could induce pluripotency.

hiPSC generation and validation. In 2007 this same group reported the successful induction of human pluripotent stem cells using similar methods (21). Other research groups experienced similar success at this time. In both Takahashi studies, extensive post induction testing was carried out to validate the induced pluripotency. RT-PCR demonstrated that these cells possessed pluripotency markers typical of ESCs (20) (21). Gene expression was analyzed using a DNA microarray, which showed that expression patterns between iPSCs and ESCs were highly similar (20) (21). Teratoma and embryoid body formation also proved that cells possessed pluripotency (20) (21). Southern and western blots were conducted and gave evidence that the cells were pluripotent stem cells as well (20) (21). hiPSC morphology, proliferation, surface antigens, gene expression, telomerase activity, and epigenetics are all highly similar to hESCs as well (22). The induction methods and verification methods conducted in these two studies are still common methods of creating iPSCs, but some variations do occur.

Additional hiPSC derivation procedures. Besides retroviral transduction, lentivirus, adenovirus, plasmid transfection, transposon, and recombinant protein methods have been described to create iPSCs (22). Other combinations of transcription factors are also used to generate iPSCs, and some have even demonstrated higher efficiency than the traditional four (22). Refer to Table 1 for a list of some iPSC generation methods.

Table 1. The Many Ways of Reprogramming Methods to Make iPS Cells

Genes	Strategy	Species	Cell Type	Efficiency	Years
Retrovirus					
Four factors (Oct4, Sox2, Klf4, c-Myc)	retrovirus	mouse	embryonic fibroblasts	0.1%	2006
Four factors (Oct4, Sox2, Klf4, c-Myc)	retrovirus	human	fibroblasts	0.01%	2007
Three factors (Oct4, Sox2, Klf4)	retrovirus	mouse	fibroblasts	0.01%	2008
		human	fibroblasts	0.001%	
Two factors (Oct4, Klf4 or c-Myc)	retrovirus	mouse	neural stem cells	0.14%	2008
Mir-302	retrovirus	human	skin cancer cells	NA	2008, Aug
Two factors (Oct4, SOX2) + VPA	retrovirus	human	fibroblasts	0.001%	2008, Oct
One factors (Oct4)	retrovirus	mouse	neural stem cells	0.1%	2009
Adenovirus					
Four factors (Oct4, Sox2, Klf4, c-Myc)	adenovirus	mouse	hepatocyte	0.0006%	2008, Nov
Lentivirus					
Four factors (Oct4, Sox2, NANOG, and LIN28)	lentivirus	human	fibroblasts	0.01%	2007
Six factors (Oct4, Sox2, Klf4, c-Myc, NANOG, and LIN28)	lentivirus	human	newborn foreskin fibroblasts	0.1%	2008
Plasmid transfection					
Four factors (Oct4, Sox2, Klf4+c-Myc)	plasmid transfection	mouse	embryonic fibroblasts	0.0015%	2008, Oct
Six factors (Oct4, Sox2, Klf4, c-Myc, NANOG, and LIN28)	plasmid transfection	human	human foreskin fibroblasts	0.1%	2009, May
Transposon					
Four factors (Oct4, Sox2, Klf4, c-Myc)	piggyBac transposition	mouse	embryonic fibroblasts	NA	2009, Apr
Recombinant protein					
Four factors (Oct4, Sox2, Klf4, c-Myc)	recombinant protein	human	fibroblasts	0.001%	2009, Jun

Table 1. iPSC Generation Methods

Table 1 was obtained from source 22. This lists several of the reprogramming methods used today to generate iPSCs. Different variations of transcription factors as well as different transduction strategies have been utilized to generate iPSCs with different levels of efficiency. This is not intended to be an exclusive representation of iPSC generation methods.

No matter the methods used, the ability to create pluripotent stem cells from somatic cells is a significant scientific accomplishment. Although other types of pluripotent stem cells have been created using methods such as somatic cell nuclear transfer (SCNT) and cell fusion, none possess the therapeutic potential that iPSCs do (23). In fact, a biological case may be made that iPSCs have the most therapeutic aptitude

out of all pluripotent stem cells including ESCs.

Biological Grounds for hiPSC Utilization

hESCS and hiPSCs have great potential for future use in the medical field, but hiPSCs have multiple biological advantages over hESCs. These are a result of hiPSCs being an autologous source of pluripotent stem cell. The autological characteristics of hiPSCs make them better suited than hESCs for clinical drug testing, disease modeling, and cell mediated therapy. Since hiPSC research is still in its infancy, it still possesses some problems such as tumorigenicity, epigenetic memory, and possible immunogenicity. These potential obstacles require more research before hiPSCs can be used in a clinical setting, but the potential of hiPSCs for medicinal use demands that more research be conducted to address these concerns.

Drug Testing and Disease Modeling

The concept of using pluripotent stem cells in drug testing and disease modeling is a fascinating one. By definition, pluripotent stem cells are able to differentiate into any type of somatic tissue. For drug testing and disease modeling, this has tremendous implications. Many tissues, such as neurons, are difficult to access, so the ability to generate these cells *in vitro* will provide great insight into the pathophysiology of diseases in these difficult access tissues and will allow for a greater amount of pharmacological testing (23).

The hiPSC advantage. Although hESCs have demonstrated the ability to differentiate into multiple cell lineages and pathologies, hiPSCs have a distinct advantage over hESCs when it comes to disease modeling and drug testing (23). hESCs have demonstrated the ability to differentiate into any adult cell type, including neurons,

cardiomyocytes, and hepatocytes. Although this has provided a great tool for drug discovery, most hESC represent generic cells that are not indicative of specific clinical conditions (24). The ability to generate a pluripotent cell line directly from a specific patient afflicted with a disease should allow genetically identical cell types from all the major organs of interest (25). Rather than putting a patient at risk by subjecting him or her to specific unknown effects of different medications in the treatment of disease, the pharmacological treatments could be tested *in vitro* on genetically identical hiPSCs from the patient. This concept could also be applied to the development of new drugs. This idea has great appeal in theory, but is it possible to carry out in reality? Recent research points to this being so.

hiPSC disease modeling research. Recent studies have described the successful generation of hiPSC lines from patients with a myriad of pathological problems (25). The most effective modeling studies have come from early onset diseases caused by strong genetic factors in a highly defined cell or tissue type (24). To date most of these studies have focused on monogenic diseases to prove that disease modeling pluripotent stem cells can be created (23). As seen in Table 2, many more disease lines have been developed using hiPSC technology instead of hESCs. In most of these studies, *in vitro* differentiation of hiPSCs to each cell type for the disease has been reported, and many studies suggest that patient specific hiPSCs demonstrate certain disease characteristics (25). For example, in hiPSCs derived from spinal muscular atrophy (SMA) patients, a progressive loss of motor neurons was noted during *in vitro* differentiation (26). This may reflect the developmental loss of motor neurons during the *in vivo* course of this disease.

Disease	Molecular defect	Phenotype demonstrated
Human ESCs		
Alport syndrome	Mutation in *COL4A5*	Not determined
Androgen insensitivity syndrome	Deletion of androgen receptor gene	Not determined
Fabry syndrome	Mutation in *GLA*	Not determined
Fanconi anaemia (carrier)	Mutations in *FANCA*	Not determined
Marfan syndrome	Mutation in *FBN1*	Not determined
Multiple endocrine neoplasia type 2A	Mutation in *RET*	Not determined
Myotonic dystrophy	Trinucleotide expansion in *DMPK* or tetranucleotide expansion in *CNBP*	Decreased expression of two members of the SLITRK family; altered neurite outgrowth, neuritogenesis and synaptogenesis in motor neuron and muscle cell co-cultures
Neurofibromatosis type 1	Point mutation in *NF1*	Not determined
Saethre–Chotzen syndrome	Mutation in *TWIST*	Not determined
Spinocerebellar ataxia type 2	Trinucleotide expansion in *ATXN2*	Not determined
X-linked myotubular myopathy	Mutation in *MTM1*	Not determined
Human ESCs and iPSCs		
Becker muscular dystrophy	Mutation in dystrophin gene	Not determined
Cystic fibrosis	Mutations in *CFTR*	Not determined
Duchenne muscular dystrophy	Mutation in dystrophin gene	Loss of dystrophin expression in muscle tissue derived from diseased iPSCs; restored by human artificial chromosome-mediated dystrophin expression
Fragile X syndrome	Trinucleotide (CGG) expansion, silencing of *FMR1*	Not determined
Gaucher's disease	Point mutation in β-glucocerebrosidase	Not determined
Huntington's disease	Trinucleotide expansion in huntingtin gene	Enhanced caspase activity following growth factor withdrawal in iPSC-derived neurons from patients
X-linked adrenoleukodystrophy	Mutation in *ABCD1*	VLCFA levels increased in iPSC-derived oligodendrocytes; reduced after treatment with lovastatin or 4-phenylbutyrate
iPSCs		
ADA–SCID	Mutations in *ADA*	Not determined
Atypical Werner syndrome	Mutation in *LMNA*	Nuclear membrane abnormalities, increased senescence and susceptibility to apoptosis observed in iPSC-derived fibroblasts
β-thalassaemia	Deletion in β-globin gene	Not determined
Crigler–Najjar syndrome	Mutation in *UGT1A1*	Not determined
Type 1 diabetes	Multifactorial; unknown	Not determined
Down syndrome	Trisomy 21	Not determined
Dyskeratosis congenita	Mutations in *DKC1*, *TERT* or *TCAB1*	Progressive telomere shortening and loss of self-renewal of iPSCs
Dystrophic epidermolysis bullosa	Mutations in *COL7A1*	Lack of expression of type VII collagen, restored following gene correction; no difference between diseased and control formation of three-dimensional skin equivalents
Familial amyotrophic lateral sclerosis	Mutation in *SOD1* or *VAPB*	Reduced levels of VAPB in fibroblasts, iPSCs and motor neurons derived from patients with VAPB mutation
Familial dysautonomia	Mutation in *IKBKAP*	Decreased expression of genes involved in neurogenesis and neuronal differentiation; defects in neural crest migration
Familial hypercholesterolaemia	Mutation in gene encoding LDL receptor	iPSC-derived hepatocytes have an impaired ability to incorporate LDL

Disease	Molecular defect	Phenotype demonstrated
iPSCs (cont.)		
Glycogen storage disease type 1A	Deficiency in glucose-6-phosphate	Hyperaccumulation of glycogen
Gyrate atrophy	Mutation in *OAT*	Not determined
Hereditary tyrosinaemia type 1	Mutation in fumarylacetoacetate hydrolase	Not determined
Hutchinson–Gilford progeria syndrome	Mutations in *LMNA*	Accelerated cell senescence, progerin accumulation, DNA damage, nuclear abnormalities, inclusions in VSMCs; phenotype corrected by HDAd-based gene repair
Inherited dilated cardiomyopathy	Mutation in *LMNA* causing *LMNA* haploinsufficiency	Nuclear membrane abnormalities, increased senescence and susceptibility to apoptosis in iPSC-derived fibroblasts
Lesch–Nyhan syndrome (carrier)	Heterozygosity of *HPRT1*	Not determined
Long QT syndrome	Mutation in genes encoding KCNQ1 or KCNH2	Arrhythmogenicity in cardiac cells; treatment with ranolazine rescues arrhythmia
MPS type I (Hurler syndrome)	*IDUA* deficiency	Not determined
MPS type IIIB	Defective α-*N*-acetylglucosaminidase	iPSCs and differentiated neurons derived from patients show defects in storage vesicles and Golgi apparatus
Parkinson's disease	Unknown or mutations in *LRRK2* or *PINK1*	Impaired mitochondrial function in PINK1-mutated dopaminergic neurons, corrected by lentiviral expression of PINK1; sensitivity to oxidative stress in LRRK2-mutant neurons
Polycythaemia vera	Heterozygous point mutation in *JAK2*	Enhanced erythropoiesis
Progressive familial hereditary cholestasis	Unknown	Not determined
Retinitis pigmentosa	Mutations in *RP1*, *RP9*, *PRPH2* or *RHO*	Decreased numbers of differentiated rod cells and expression of cellular stress markers
Rett syndrome	Mutation in *MECP2*	Decreased synapse number, reduced number of spines and elevated LINE1 retrotransposon mobility
Schizophrenia	Unknown	iPSC-derived neurons from patients show diminished neuronal connectivity and decreased neurite number, PSD95 and glutamate receptor expression; neuronal connectivity is improved following treatment with loxapine
Scleroderma	Unknown	Not determined
Shwachman–Bodian–Diamond syndrome	Mutation in *SBDS*	Not determined
Sickle cell anaemia	Mutation in *HBB*	Not determined
Spinal muscular atrophy	Mutation in *SMN1*	Reduced SMN levels in iPSCs, reduced size and number of motor neurons; valproic acid and tobramycin increases the number of SMN-rich structures (gems) in iPSCs derived from patients
Wilson's disease	Mutations in *ATP7B*	Mislocalization of mutated ATP7B and defective copper transport in iPSC-derived hepatocyte-like cells; rescued by lentiviral gene correction or treatment with the chaperone drug curcumin
X-linked chronic granulomatous disease	*CYBB* deficiency	Lack of ROS production in neutrophils, corrected by insertion of *CYBB* minigene
α1-antitrypsin deficiency	Mutation in α1-antitrypsin	α1-antitrypsin polymerization

Table 2. hESC and hiPSC Disease Models

Table 2 was obtained from source 24. This represents a current compilation of hESC and/or hiPSC lines derived from patients with the specified diseases. The molecular defect and phenotype of each disease is provided if previously determined.

Another study demonstrated that cardiomyocytes derived from hiPSCs of patients suffering from LEOPARD syndrome were larger than normal, which may be reflective of the hypertrophic cardiomyopathy typical of this pathology (27). Similarly, gluatamatergic neurons with less synapses and diminished calcium transients than controls were seen in hiPSCs derived from methyl CpG binding protein 2 (MeCP2)-deficient females with RETT syndrome, which is typical of cells from RETT patients (28). A number of other studies have yielded similar results in which hiPSCs derived from diseased patients exhibit cellular disease characteristics (24). In some of these studies, such as in the described studies of SMA and RETT syndrome hiPSCs, known treatments for these diseases were used on the hiPSC models and brought healing of these diseased cells (25).

Although the successful production of disease phenotypes from hiPSCs is a major accomplishment, the ultimate goal of disease modeling and drug therapy is to develop treatment based upon these diseased hiPSC lines (25). Besides modeling diseases for drug discovery, hiPSCs are thought to potentially serve as a model for pre-symptomatic abnormalities in patient derived cells (25). This could provide insight into disease onset as well as be used in the development of early diagnostic tools and drug therapy (25). Due to the infancy of hiPSC modeling, no new drugs have been discovered yet to reverse disease phenotypes (neither has this occurred from hESC modeling) (25). However, hiPSCs provide an unmatched prospective use in drug testing.

hiPSC drug testing potential. There is a high drug attrition rate in the conventional method of drug testing. This is because these drugs cannot be tested in a relevant human diseased population until late in the drug testing process (24). Most clinical trials fail due to toxicological or efficacy issues and many positive results from

preclinical testing phases do not transfer to clinical testing because of highly engineered human cell lines that create imperfect modeling systems (24). Genetic variations in humans, such as millions of single nucleotide polymorphisms, copy number variations, deletions, insertions, inversions, and epigenetic differences make it difficult to determine pharmacological effects in *in vivo* models (24). This issue could become obsolete with the implementation of patient-derived hiPSCs for drug testing. It is important to acknowledge that hESCs would be a poor choice for drug testing due to the same genetic problems that face adult human cell lines.

Given their identical genetic makeup with their origin, hiPSCs would be an ideal source for preclinical drug testing. The efficiency of clinical trials could be increased using patient-derived hiPSCs because the actual diseased cells from a large number of patients could be tested *in vitro* for efficacy, toxicity, and dose response (24). Several other benefits to this proposition would result as well. Patient compliance issues and patient risks would be largely eliminated using this method of drug testing. The created hiPSC lines could be used to test many future potential drugs as well (24). Additionally, the hiPSC lines could be used to study the effect of the potential drugs in all tissue types after differentiation and represent a “patient in a dish” (24). The potential for hiPSCs in drug testing and drug discovery through disease modeling is remarkable. hESCs are not capable of such potential due to their nonspecific genetic makeup. For a schematic representation of the proposed use of hiPSCs in drug screening and disease modeling, see Figure 1. hiPSCs not only possess an exclusive extreme potential in drug testing and creation, but also in cell-mediated therapy.

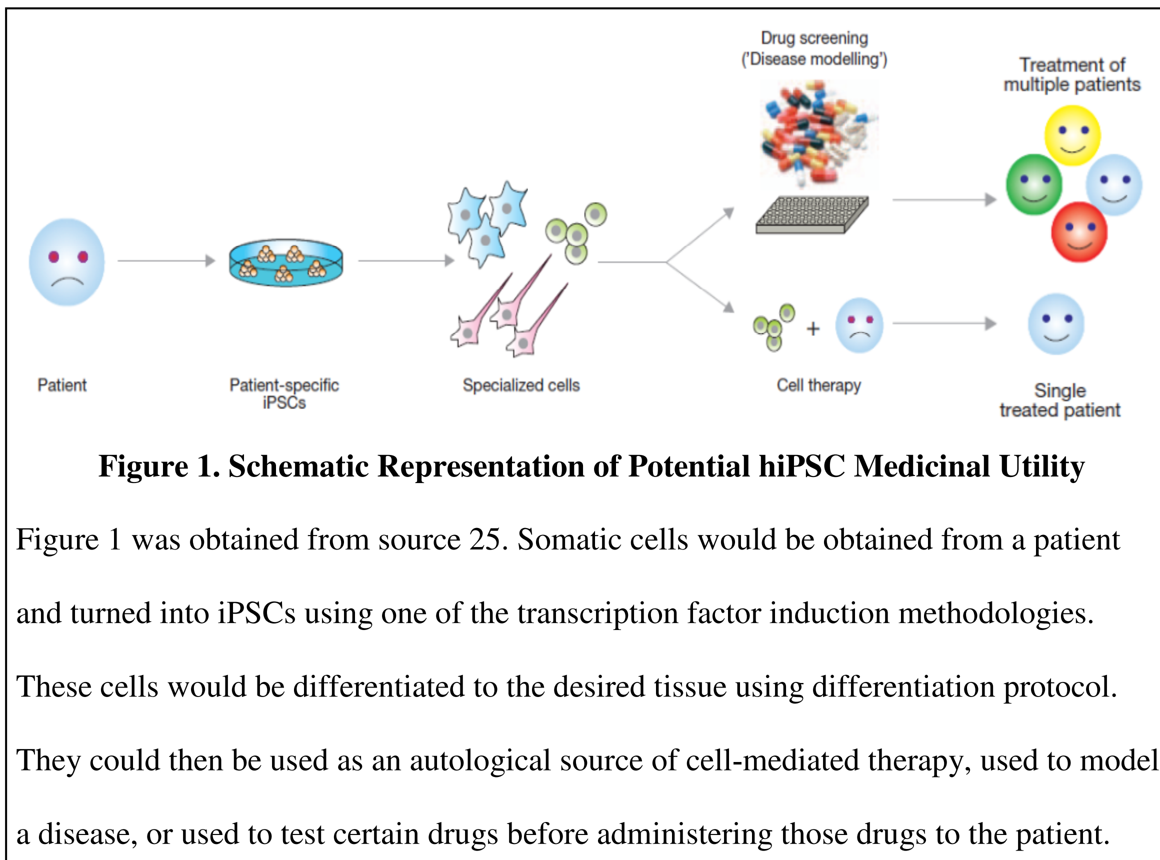

Figure 1. Schematic Representation of Potential hiPSC Medicinal Utility

Figure 1 was obtained from source 25. Somatic cells would be obtained from a patient and turned into iPSCs using one of the transcription factor induction methodologies. These cells would be differentiated to the desired tissue using differentiation protocol. They could then be used as an autological source of cell-mediated therapy, used to model a disease, or used to test certain drugs before administering those drugs to the patient.

Cell-Mediated Therapy

Cell-mediated therapy is the repair or replacement of damaged or poorly functional tissue with new cells. The idea is that healthy cells will be able to correct whatever underlying abnormality may be present in the dysfunctional cells or possibly replace the dysfunctional cells altogether. There are ASC-mediated therapies being used today, such as hematopoetic stem cell transplantation for patients with blood or bone marrow cancers. Although these ASCs have proved valuable, hiPSCs possess the most potential in cell mediated therapy, even more so than hESCs.

hiPSC advantage in cell-mediated therapy. Pluripotent stem cells hold a theoretical advantage over ASCs in the area of cell-mediated therapy due to a couple different factors. First, pluripotent stem cells have the potential to be used for a broader

range of target tissues due to their enhanced differentiation potential than the typically multipotent ASCs. Secondly, the pluripotent stem cells are able to be differentiated into cell types that would be difficult to obtain from an adult source, such as neurons or cardiomyocytes. This is one of the reasons why relatively easy access tissues, such as hematopoetic and skin ASCs, are nearly exclusively used in adult stem cell-mediated therapy (25). Although both hESCs and hiPSCs seem to possess greater biological potential for cell-mediated therapy than ASCs, hiPSCs are clearly a better option for future utilization in cell-mediated therapy than hESCs.

Just as the autologous nature of hiPSCs proved to distinguish them as superior to hESCs in drug testing and disease modeling, this nature makes hiPSCs a better alternative than hESCs in the area of cell-mediated therapy. One of the greatest difficulties facing regenerative medicine is immune rejection. hiPSCs are able to bypass this difficulty by creating a self-source for transplantation and thereby allow for the possibility of a “personalized” approach to cell-mediated therapy. For example, fibroblasts could be obtained from a patient with ischemic heart disease. These fibroblasts could be reverted to hiPSCs after the introduction of Oct3/4, Klf4, Sox2, and c-Myc. The hiPSCs could then be differentiated into cardiomyocytes and injected into the ischemic areas of the heart, thereby causing improved cardiac functioning without eliciting an immune response. Refer to Figure 1 for a schematic representation of this proposed process of cell-mediated therapy. In contrast to hiPSCs, hESCs generated for cell-mediated therapy would possess immunogenicity, and would therefore be a poorer option than hiPSCs. Recent research has shown significant progress in making hiPSC cell-mediated therapy a reality.

hiPSC research and potential in regenerative medicine. hiPSC cell-mediated therapy is no longer strictly theoretical. Multiple studies have demonstrated improvement of the diseased specimens after iPSC transplantation. In one such study, hematopoietic cells generated from murine iPSCs were shown to help improve a humanized mouse model of sickle cell anemia (25). In this study a transgenic mouse with a mutation in the human hemoglobin sequence received correction of this mutation through homologous recombination. iPSCs were derived from this mouse and were differentiated into hematopoietic progenitor cells *in vitro.* These cells were then transplanted back into the transgenic mouse, which resulted in restoration of normal hemoglobin levels and an improved erythrocyte phenotype (25).

iPSCs have also demonstrated improvement in cell-mediated therapy in harder access tissues like central nervous system (CNS) cells. Rats with Parkinson's disease have shown improvement after receiving neurons derived from iPSCs. Likewise, glial cells derived from iPSCs have demonstrated the ability to help protect neurons after transplantation (29). Cardiac iPSC cell-mediated therapy has shown promise as well. One study delivered autologous murine iPSC-derived cardiomyoctyes into the myocardium of mice with infarcted hearts. These iPSC-derived cardiomyoctes replicated without disrupting neighboring cell structure and improved contractile performance, ventricular wall thickness, and electrical stability of the infarcted hearts. Cardiac muscle, smooth muscle, and endothelial tissues were also regenerated in this study (30). Other studies have demonstrated the possibility of creating a biological pacemaker utilizing iPSC cell-mediated therapy (31).

Like numerous other studies, the studies mentioned above show that iPSC cell-mediated therapy can lead to improvement in diseased tissues. Besides implantation into diseased tissues, one of the chief aims of hiPSC research is to develop methods to create entire organs for organ transplantation. Complex organs such as the brain, heart, and liver have very complicated anatomy consisting of multiple cell types and precise chemical consistencies that are vital to their proper functioning (32). Because of this and the fact that hiPSC research is at its infancy, the creation of organs with hiPSCs is likely many years away. Although organ generation is distant, progress has been made in making organ generation a greater possibility. Successful techniques have been developed for angiogenesis in small organs and tissue, which is vital for the generating organs from hiPSCs (32). Genetically human organs could theoretically be created in animals considering recent advances in scientific technology. Other propositions for the future of organ generation include "bioprinting" of organs by using a 3-D printer that uses hiPSCs as the "ink". After programming the printer with the right anatomical structure, the correct type of hiPSCs could be placed in their corresponding location (32). No matter the difficulty, the possibility of creating an entire organ from an autological source is extremely appealing and could have a tremendous positive impact on the future of medicine. This is one more reason why hiPSC research should be conducted all the more vigorously and hESC research be put to a halt.

Biological Concerns with hiPSCs

Although hiPSCs have shown extraordinary potential in medicine, there are still some concerns raised about these cells. hiPSC research has only been around for less than a decade, so many of the concerns raised may simply require more research in the years

to come to adequately assess if the concerns may have any negative consequences. It should be noted that some of the concerns raised about hiPSCs, such as tumorigenicity, are also major problems with hESCs. The main issues that will be addressed below include this issue as well as concerns about epigenetic differences, immunogenicity, and induction methods.

Innate tumorigenicity. One of the consistencies among all pluripotent stem cells, including hESCs and hiPSCs, is their ability to generate teratomas in suitable hosts. In fact, most pluripotent characterization procedures involve delivering pluripotent stem cells into SCID mice to measure for teratoma formation. Positive teratoma formation is used to designate successful generation of pluripotent stem cells. This information alone demonstrates the close relationship between pluripotency and tumorigenicity, and it is therefore no surprise that tumorigenicity is one of the major obstacles to overcome for hiPSCs and hESCs (33). Although much more research needs to be conducted to allow for the elimination of tumor formation after pluripotent stem cell transplantation, recent studies have shed light on some of the possible ways to minimize the tumorigenesis in cell-mediated therapy.

A recent study demonstrated that the somatic origin of iPSCs is crucial in determining the level of tumorigenicity. In this study, murine iPSCs derived from adult mouse tail-tip fibroblasts had the highest propensity of tumorigenesis, while those from stomach tissues had the lowest propensity (34). It is proposed that some tissues, like the fibroblasts, may lead to iPSCs that are resistant to differentiation and are teratoma-initiating (34). Other studies have demonstrated that non-differentiated iPSCs are more tumorigenic than differentiated iPSCs. Hattori et al. created a cardiomyocyte purification

methodology that selects pluripotent stem cell-derived cardiomyoctes against undifferentiated pluripotent stem cells at >99% purity. These cardiomyocytes are non-tumorigenic after transplantation into SCID mice, demonstrating that transplantation that utilizes differentiated pluripotent stem cells could be the future direction for stem cell-mediated therapy (35).

Besides somatic origin and purification of differentiated iPSCs, immunity plays a large role in the amount of tumorigenesis after pluripotent stem cell transplantation. The amount of tumorigenesis after pluripotent stem cell transplantation is remarkably reduced in immunocompetent specimens compared to SCID specimens (33). It has therefore been deduced that the immune system plays a major role in regulating tumorigenesis, and research has supported this notion. The presence of NK cells and the complement system, as well as certain cells in the adaptive immune response, have shown the ability to limit the amount of tumorigenesis in the stem cell recipient (33). Other factors that play a role in the amount of tumorigenesis include the amount of pluripotent stem cells transplanted and the location of the grafting site in the recipient (33).

It is again important to note that despite the difficulties hiPSC may face in the issue of tumorigenicity, hESCs face the same problem. hiPSCs have actually been proposed to be a better source of pluripotent stem cells than hESCs for cell-mediated therapy because of their ability to recognize self and non-self. The self cells (the differentiated hiPSCs) would be able to be distinguished from tumor forming cells by the host. The host's immune system would be able to help extinguish these tumor-forming cells and allow the differentiated hiPSCs to proliferate (33). Despite the research

mentioned above, much more research needs to be conducted on eliminating teratoma formation before hiPSCs can be used for human cell-mediated therapy.

Possible immunogenicity. As has been stated numerous times throughout this work, the major biological appeal of utilizing hiPSCs in place of hESCs lies in the fact that hiPSCs would provide an autologous source. In theory, this autological source should not provoke an immune response in possible cell-mediated therapy procedures. However, one recent study suggested that iPSCs may possess immunogenicity. This claim was made after examining transplantation of undifferentiated mouse iPSCs and assays for teratoma formation (36). Some possible explanations as to why immunogenicity was observed are the expression of human leukocyte antigens (HLAs) or epigenetic memory of iPSCs, which will be discussed shortly (37). Although this study may seem to support the notion that iPSCs possess immunogenicity, this study was conducted on undifferentiated iPSCs. Numerous studies have been published on transplantation of differentiated iPSCs without provoking an immune response (34). These results suggest that non-differentiated iPSCs have different cellular properties than differentiated iPSCs and that the selection of properly differentiated iPSCs will reduce the risk of immunogenicity. Along with the decreased likelihood of tumorigenesis, this demonstrates that differentiated iPSCs may be the ideal candidate for future cell-mediated therapy.

Epigenetic memory and genetic differences. Comparative studies of ESCs and iPSCs have demonstrated that these two stem cell types are extremely similar and nearly indistinguishable (25). Although this is true, there is evidence that there are subtle differences between the two, particularly epigenetic differences. Recent studies have shown that some iPSCs retain some epigenetic properties of their somatic cell origin.

These epigenetic factors include either DNA methylation or histone modification (38). However, most of the retention of these epigenetic similarities is found in early passage numbers of iPSCs, and can therefore be considered as transient epigenetic memory (38). The cells that display epigenetic memory have also shown greater success in differentiating into the cells of the original somatic tissue, which would make them better candidates than hESCs in cell replacement therapy (39).

In addition to epigenetic memory differences from ESCs, some iPSCs have possessed genetic mutations including insertions, deletions, and point mutations (38). Some of these have been predicted to alter protein function. Although hESC research advocates may be quick to argue that this is due to inherent differences between hESCs and hiPSCs, they are likely due to many different factors. Some of these mutations were already present in the somatic donor cells. This points to the discrepancy that the hESCs used in many of these studies were most likely originated from multiple embryonic founder cells, which were compared to hiPSCs from donors with an unknown genomic integrity (25). Some of these mutations were also present in late-passage hESCs (38). Other mutations could have arisen from simple *de novo* replication. The most probable explanation is that these mutations arose from unpolished hiPSC generation procedures. Recent studies have shown that genetic background, lab-to-lab variation, passage number, and the use of viral integration have a significant effect on gene expression of hiPSCs (25).

Improved hiPSC generation methods should limit the amount of mutations present in hiPSCs. The use of retroviral transduction in hiPSC generation has been raised as a concern by many due to the possibility of causing mutations and cancer. In response to

these concerns, research has been conducted to create new methods of hiPSC generation without viruses by utilizing micro-RNA, transposons, and recombinant proteins (refer to Table 1 for the iPSC generation methods) (22). New research will also lead to more efficient hiPSC induction methods, which has been another concern associated with hiPSCs. Although epigenetic and genetic concerns have been raised about hiPSCs, there has not been substantial research to support the notion that these differences from hESCs will lead to any problems (25). However, careful examination of these differences is warranted before hiPSCs are to be used in cell-mediated therapy since hiPSC research is still at its beginning.

Ethical Grounds for hiPSC Utilization

The ethical controversy surrounding hESC research is the largest problem hESC research faces. This is ultimately the most significant reason why hESC research should be eradicated and hiPSC research should be conducted more intensely. hESC research proponents cite a number of arguments in favor of hESC research, but these arguments are rationally and morally flawed. The ethical arguments against hESC research hold greater weight.

Ethical Arguments in Favor of hESC Research

Although there have been many ethical arguments developed in favor of hESC research, two of the main arguments will be addressed here. These are the imperative to end suffering and the non-personhood of the embryo (40).

The imperative to end suffering. This argument holds that hESC research is necessary to aid in ending the suffering of millions around the world who suffer from illness and disease (41). It is argued that since there are millions of people suffering from

thousands of different debilitations, it is mankind's moral responsibility to do what it can to relieve this suffering. hESCs are believed to be able to aid in this matter. Therefore, many advocates of this position would state that hESC research is a necessity (41). This argument clearly motivated President Obama to lift the ban on federal funding on hESC research. This is seen in his speech following the revoking of this ban when he stated, "Scientists believe these tiny cells may have the potential to help us understand, and possibly cure, some of our most devastating diseases and conditions. . . I believe we are called to care for each other and work to ease human suffering. I believe we have been given the capacity and will to pursue this research – and the humanity and conscience to do so responsibly." (42).

The non-personhood of the embryo. The most important issue encompassing the hESC research debate is the status of personhood of the embryo. In this second argument examined in favor of hESC research, proponents would state that at the blastocyst stage from which hESCs are derived, the blastocyst is not a human person (40). In this view some hold that the human embryo is degraded to nothing more than a clump of cells (41). Advocates for hESC research would then propose that an embryo possesses a lesser moral status than adult humans, and therefore it is justifiable to kill them for research (43). When coupled with the argument of the imperative to end suffering, this leads to a natural sense of obligation to conduct hESC research.

Others, even though they do not believe the embryo is a human person, believe that because it has the potential to become a person it deserves "respect" or "profound respect" and its life should not be destroyed without a just cause (44). The just cause, they propose, is the imperative to end suffering, and since hESC research could help end

that suffering, the destruction of the embryo is justified for hESC research. This is known as the sacrifice argument (40). This argument states that since these embryos are destined to be discarded and not used in IVF, why not use them in research to help relieve suffering? If hESC research is able to bring a sufficient measure of good despite the destruction of embryos, then it is justifiable. This argument is made on direct consequentialist grounds and is used by proponents of stem cell research to bypass the moral status of an embryo (43). They reason that if these embryos could be used in research, nothing would be lost in destroying them, and something could potentially be gained (41).

Many proponents of hESC research do not believe that embryos should be created solely for the purpose of being destroyed for research. However, since embryos created during IVF are going to die anyways without implantation and further development, they should be used for hESC research. This is referred to as the "nothing is lost" principle, and it is one of the strongest arguments in favor of hESC research. This principle states that one may justifiably directly kill another innocent human being when that human being is going to die anyways and another innocent life may be saved (40). Some advocates of hESC research may grant that the human embryo is a human person. However, they state that the embryo has no inherent right to gestation and life. They equate this to the absence of a developed human's right to social protection from diseases and disasters (41).

Ethical Arguments against hESC Research

No matter the biological case for hiPSC research in place of hESC research, by far the strongest case against hESC research is the ethical one. This is due to the

necessary destruction of a living human embryo in order for hESC to take place. Even if hESCs were a better biological source of pluripotent stem cells than hiPSCs, hESC research should be brought to a halt because of its requirement to destroy a human embryo. The most important question surrounding the stem cell debate still lingers: Is a human embryo a human person? Several strong arguments exist to demonstrate that this is so.

The personhood of the embryo. If a human embryo is in fact a human person, then hESC research renders the killing of a human person as morally permissible (40). A stronger case exists for the personhood of the embryo versus the non-personhood of the embryo.

Definition of a person. What constitutes personhood? There are two main approaches to this position: the functionalistic and the essential. The functionalistic view holds that an entity is a person only if it functions as a person in aspects of consciousness, rational thinking, and self-awareness. This view produces false regulations on personhood. Under the functionalistic definition of personhood, those who are sleeping or under general anesthesia would not be classified as a person. The essential view provides a better alternative to defining persons.

Under this view, an entity is a person if it possesses the nature of a person. In other words, it possesses the basic inherent capacity to function as a person even if this capacity is never actualized. The human embryo is a human person with a continuous history. Ontologically, essence precedes functioning. In order for something to be able to act like a person it must in fact be a person. Since a human embryo possesses the basic inherent capacity of a human person, it is therefore a human person.

Beginning of personhood. There are many different thoughts on when personhood begins, and these thoughts have implications on whether one believes an embryo is a person. Some of these views include the points of conception, implantation, brain development, appearance of humanness, sentience, quickening, viability, birth, and gradualism. Not all of these views will be addressed, but using the essential view of personhood, conception appears to be the beginning of personhood. Throughout the development of a human organism, there is no equally decisive break than at the moment of conception (44). Prior to conception, a haploid sperm and ovum containing separate information exist. After conception, a genetically unique diploid organism comes into existence. The other views on the beginning of personhood fail to ascribe personhood to the zygote even though this is the moment where the basic inherent capacity of personhood is initiated. The zygote is an integrated, self-developing organism capable of the progressive development that is characteristic of human life (44). Since personhood is dependent on essence and personhood begins at conception, the destruction of an embryo for hESC research is to be equated with the destruction of a human person. A human embryo is not simply a potential person, but it is already in essence a person with potential (40).

Objections to the sacrifice argument. As discussed previously, there are some who believe that although a human embryo is not a human person it should be treated with some measure of respect, even “profound respect” (40). Because of this, its life should not be casually discarded of, but it may be “sacrificed” for a just cause (40). This argument possesses several flaws.

Benefit of the doubt. By stating that a human embryo needs to be treated respectfully, this position would necessarily acknowledge that the human embryo could in fact be a person. By no means can the claim be made that the embryo is absolutely not a person (40). Since an embryo will develop as a human person if it is not interfered with, the burden of proof lies on those who hold to the non-personhood view of an embryo. This is regarded as the benefit of the doubt argument (40). Even if one believes that the embryo is not a person, one cannot be completely sure of this. Therefore, the destruction of an embryo is at best criminal negligence.

False respect for the embryo. The measure of genuineness of those who state they have "profound respect" for an embryo they intend to kill must be called into question. In harvesting hESCs, one is knowingly destroying the human embryo. It is difficult to justify the destruction of an undisputed potential human being while making the claim that the embryo is being respected (40). One must not forget that the embryos are destined to die based on the researcher's volition (44). One cannot pretend that the death of the embryo is a natural event unaffected by man's choices. Although these embryos may not have directly been chosen to be created by the researchers destroying them, the researcher's relationship to their death is not any different from a moral standpoint (44). Up to 38% of all frozen embryos are simply discarded, demonstrating that these embryos are not even treated respectfully by the hESC advocates' own definition (45).

Pseudo-sacrifice. The term "Sacrifice Argument" is a misleading one. Sacrifice necessitates willingness on the part of the party that is standing something to lose (40). Sacrifice is a noble act, but the volition of the effected party is necessary in order to

constitute as sacrifice. One can choose to sacrifice his or her own life but not the life of another individual. “Sacrificing” another’s life is equivalent to murder (40). Even if that person thinks sacrificing another’s life is justifiable, it is still murder nonetheless. Since the embryo is not voluntarily laying its life down for the research of diseases, the act of destroying the embryo may not be called sacrifice (40).

Is relieving suffering a just cause? The notion of relieving suffering as means to justify the destruction of human embryos needs to be called into question. Suffering is a subjective item. Proponents of hESC research say that embryo destruction is justified based on the suffering of millions worldwide, but what if there were only a few people suffering? Can embryos be killed for them? The level of suffering necessary to justify killing embryos is subjective, and the end to killing embryos for suffering may never cease (40). If the destruction of human embryos could provide the benefit of creating a new antiwrinkle cream to relieve the “suffering” associated with aging, would hESC destruction not be justified under this argument (43)? This notion seems absurd, and for good reason. hESC research has produced no treatment options to date, yet countless numbers of human embryos are destroyed on a regular basis. Even though the desire to minimize suffering is a noble one, man is not required to use any means necessary to do so (40).

Objections to the nothing is lost principle. The nothing is lost principle states that it is justifiable to kill innocent human embryos since they are going to be destroyed anyways and their destruction may result in saving another innocent life (46). A recent estimate revealed that there are approximately 400,000 frozen embryos in fertility clinics nationwide (46). Proponents of the nothing is lost principle would state that since these

embryos are going to be destroyed anyways, they should be used for research so some measure of good may be extracted out of them. Although this is one of the most persuasive of all the arguments in favor of hESC research, there are three objections that can be made to this argument (40).

Incomplete count. Advocates of hESC research neglect important information from the estimate that there are 400,000 frozen embryos throughout the USA. The study that unveiled this estimate also stated that only 2.8% of these 400,000 embryos are designated for research, which equates to about 11,000 embryos (46). Since most of these 11,000 embryos have been in cryopreservation for a number of years, their ability to develop into blastocysts and eventually hESC lines is greatly diminished. The estimated amount of viable hESC lines that could actually be created is 275 (46). This is not nearly the amount hESC proponents advertise.

Unjustifiable experimentation. The fact that the embryos are going to die does not warrant them to be subjected to research. If human embryos are in fact persons, then they should be respected as persons no matter what fate they may face (44). The notion that it is acceptable to experiment on weaker forms of human persons that are destined for death has been seen throughout history. Many Nazi physicians reasoned that since the Jews were going to die anyways, they wanted to create good out of the situation by experimenting on them. While researchers should not be thought of as equivalent to Nazi physicians, the principle remains the same, and experimentation on those destined for death can result in extremely negative consequences (40).

Alternative to destruction. The fact that there are some frozen embryos that will not be used in family building does not automatically mean that these embryos are

destined for destruction. Although the majority of frozen embryos are donated for research, other alternatives are available (45). One such alternative is adoption, and some choose to donate their frozen embryos to a specific couple (45).There are specially created adoption agencies for finding parents for frozen embryos (40). Embryos are not created specifically to be adopted in these agencies as this would spark more ethical debate. These agencies work to find homes for already created frozen embryos, thereby negating the presumptuous destiny for destruction (40). Although adoption is a viable alternative, only 3% of donors choose to donate their embryos in this way (45). 59% donate their embryos to research, and 38% choose to discard them (45). These latter options, especially the option of discarding, are highly irresponsible and inexcusable. The best alternative for current frozen embryos would be to offer them up for adoption in order to avoid killing these human persons.

Conclusion

There has been a large amount of debate surrounding hESC research since the creation of the first successful hESC line in 1998 after decades of previous research. Since this time, many political regulations have been implemented or rescinded, which has added to the controversy. With the advent of hiPSCs in 2007, much of this controversy can be regarded as unnecessary. hiPSCs clearly possess greater potential than hESCs in the field of medicine due to their autologous nature. hiPSCs offer the possibility of generating a “patient in a dish” to be used for drug testing and have already proven to be a more effective source for disease modeling than hESCs. Cell-mediated therapy is also better suited for hiPSCs due their lack of inherent immunogenicity unlike hESCs. Admittedly, much more research needs to be carried out to address the possible

problems of hiPSCs before they can be safely used in cell-mediated therapy just as would be the case for hESCs. hiPSCs are biologically superior to hESCs in terms of medical potential and should be researched instead of hESCs on biological grounds. Even if hiPSCs were not biologically superior to hESCs and there was no viable pluripotent alternative to hESC research, hESC research should be eradicated on ethical grounds alone. The arguments in favor of hESC research are selfish, prideful, and largely irrational. A human embryo possesses the basic inherent capacity of a human and is a human by essence. The destruction of a human embryo is therefore equivalent to murder, and because of this hESC research needs to be brought to a halt. hiPSCs should be utilized in place of hESCs for both biological and ethical reasons.

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