Introduction to somatic cell nuclear transfer (SCNT)

Somatic cell nuclear transfer (SCNT) is of particular interest to medical science and society because it affords a new opportunity to create stem cells that are genetically identical to the somatic cells of the donor.

Recent discovery in SCNT and its ethical implications

In SCNT, the genetic material of a somatic (body) cell from one individual and the enucleated egg from another, in the first days of human embryological development, together form a blastocyst capable of generating a stem cell line. As cloned cells, these stem cells could be used for a range of potential medical treatments without the risk of rejection which remains a current complication in organ transplantation.

A significant ethical issue with SCNT requires the destruction of the human embryo. (Refer back to Module 2: Introduction to stem cell bioethics) While some religious authorities view the SCNT embryo as a human life no different from a life created from sperm and egg, in reality, the only way an early SCNT embryo can develop into a fetus is if someone were to implant the pre-embryo into a human surrogate - an action the US government is not willing to condone.

A recent paper in 2013 on SCNT by Dr. Shoukhrat Mitalipov and his colleagues comes at a crucial point in the still young history of stem cell research (Tachibana, et al., 2013) Their work is a milestone discovery toward deriving human pluripotent stem cells via SCNT. They successfully fused the nucleus obtained from a baby's skin cell and applied SCNT technology to generate a human blastocyst from which they isolated and maintained human embryonic stem cells. The technical modifications presented in this paper are noteworthy for several reasons. First, previous attempts in applying SNCT to human oocytes failed because premature completion of meiosis occurred during the process resulting in the subsequent loss of the capacity of the oocyte to reprogram somatic cells to a pluripotent state. In their procedure, the authors added caffeine to the culture medium to slow meiotic completion and oocyte activation and facilitate the differentiation of the oocytes into blastocysts. Second, the authors discovered that using fetal cells as the source for donor nuclei improved the success of SCNT. Finally, the authors reported that oocytes obtained from women who produced fewer mature oocytes in response to hormonal stimulation were better suited for SCNT than oocytes obtained from women who generated many mature oocytes.
Interestingly, the authors were asked why it took so long to apply SCNT to human cells. They responded that most of the time was spent navigating United States regulations on embryo research.

The scientific motivation by Dr. Shoukhrat Mitalipov to apply SCNT to human beings was not to clone a human being. Rather, it was to obtain patient-derived embryonic stem cell lines that can be used to study and potentially treat various human diseases. In fact, their group chose to generate stem cells from a patient with a genetic defect called Leigh syndrome. These embryonic stem cells derived from the baby with Leigh syndrome will be used to consider various types of therapeutic stem cell repair without the fear of transplant rejection because the cells will be obtained from the patient. This type of stem cell application to specific patients could lead to important future treatments for neurodegenerative diseases such as Parkinson’s, Huntington’s, ALS, and Alzheimer’s, as well as heart and liver diseases.

Since this publication, two more articles have confirmed the capacity to apply SCNT to human cells. Chung et al., 2014 reported that SCNT can be used to generate embryonic stem cells from a thirty-five and seventy-five year old adult thereby avoiding the need to use fetal cells as the donor cells in SCNT. They propose that age-related changes such as shortened telomeres and oxidative DNA damage do not hinder successful reprogramming of elderly adult donor nuclei. In a second article, Yamada et al., 2014 used histone acetylation inhibitors to allow for the oocyte to remove epigenetic modifications of the donor DNA and were able to generate for the first time an embryonic stem cell line obtained from a female patient who has type I diabetes.

From a scientific perspective, this report represents a milestone in understanding of human nuclear reprogramming. On the other hand, it is clear that human SCNT research has progressed slowly because of the ethical concerns how to obtain human oocytes for these studies and where it is ethical to compensate women to donate eggs for stem cell research egg. For more information see (Hyun and Tesar 2011).

Thought questions

A constitutional amendment facing voters in Mississippi on Nov. 8, 2011, and similar initiatives brewing in half a dozen other states including Florida and Ohio, would declare a fertilized human egg to be a legal person, effectively branding abortion and some forms of birth control as murder. See: New York Times October 26, 2011.

Is an SCNT-created embryo, produced in a Petri dish using a donated enucleated oocyte and donated genetic material, biologically, ethically, and spiritually the same as creating identical twins via IVF?

The two main purposes or uses of SCNT are as follows:
1. **Research cloning** (sometimes called therapeutic cloning) involves the use of SCNT-generated stem cells for diverse experimental (e.g., stem cell research) and clinical (e.g., regenerative medicine, individualized medicine) purposes. In scientifically and ethically acceptable protocols, the embryos that are grown in a Petri dish are not maintained beyond 14 days as recommended by the National Academies of Sciences. (NAS 2005).

2. **Reproductive cloning** uses the same initial technologies, but the intent is to implant the embryo into an appropriately stimulated uterus to allow it to develop, creating a genetically “identical twin” of the somatic cell donor.

Success rates to clone animals are low for various technical reasons. Dolly, the sheep, and Snuppy, the Afghan hound, are examples of successful reproductive cloning yet many embryos were used in the process. More species of animals are now being cloned. While any attempts to clone human beings are universally considered unacceptable, specific laws against types of cloning vary from state to state and country to country. (See for example, the Human Cloning Laws set out on the Web site of the National Conference of State Legislatures, (Reproductive-Cloning 2008; Lo, Parham et al. 2010).

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**Thought question**

When we use cloning technologies, is the resulting cell line, or the resulting organism (if allowed to develop) really an exact genetic copy of the donor cell? Why or why not?

In SCNT, the donor cell contributing the genetic information (i.e., the nucleus) can be of embryonic, fetal, or adult origin. The fact that SCNT can use adult cells is one of the most innovative discoveries regarding this technology, in part because it obviates the need for sperm to generate an organism. A wide variety of differentiated adult cells, such as those obtained from skin, fat, or blood have served as donor cells for nuclei to be implanted in the enucleated oocyte.

Despite all the research, there is a wealth of biological information yet to be discovered relating to the egg’s capacity to reprogram DNA to initiate fetal development. As of 2015, the role of de-methylation of DNA or its associated proteins appears to play a major role in re-programming DNA. (see Yamaguchi, et al., 2013).
Fertilization and cloning. (Source: Nature)

One of the major clinical objectives of SCNT is to generate patient-specific stem cell lines for the development of patient-specific treatments (Chung et al., 2014). Stem cells obtained from patients with genetic-based or associated diseases can be isolated and used as screening tools to test specific treatments at the cellular level in vitro.

A second major clinical objective utilizing embryonic stem cells derived by SCNT is their use in cell replacement therapy. In cell replacement therapy, the intent is for stem cells to be obtained from patients. These cells are then genetically re-programmed to be implanted back into the patient to replace damaged or impaired tissue in disorders such as diabetes, Parkinson's disease, heart disease, as well as in spinal cord injuries (Doss, Koehler et al. 2004; Hall, Stojkovic et al. 2006). The hope is that this type of stem cell therapy will alleviate chronic, debilitating, or incurable diseases for which currently no effective therapies are available. However, any stem cell replacement protocol must consider many health risks, including the development of tumors by the implanted stem cells. Encapsulation of stem cells is an emerging technology that may significantly reduce many health risks. Encapsulation creates a delivery platform, a transitory structural support, and mechanical immune barrier, thus enabling transplantation into heterotopic sites. In addition, this mechanical barrier might also prevent the spread of any tumor cells generated from the implanted stem cells (Salvatori, 2014).

One example of a very useful cell replacement therapy would be for type 1 diabetes. The disease destroys the patient’s beta cells, which are located in the pancreas. Theoretically, somatic cell-derived stem cells from this patient could be re-programmed to produce regenerating beta cells of the islet that would generate insulin. The hope is that any potential cellular regeneration would improve the patient's health without the risk of rejection, and that the disease process would not continue to kill the regenerated beta cells. For more information read (Giannoukakis and Trucco. 2015).

Sadly, we are years away from knowing if this SCNT technology will truly be clinically effective for large numbers of people. Scientists have an important obligation to reduce hype and to help the public understand that the process of discovery is long and costly. The new technology will have to undergo the expensive and time consuming stages of Phase 1, 2, and 3 clinical trials before the FDA will grant approval of the technology.

Unlike the proposed stem cell therapy mentioned above, organ or cell transplantation that currently uses organs from surrogate donors requires that the recipient be maintained on one or more anti-rejection drugs such as cyclosporine, prednisone, azathioprine, or tacrolimus (FK506). These anti-rejection drugs often have powerful, disruptive, and debilitating effects. Cyclosporine, one of the most frequently used anti-rejection drugs, must typically be taken by a transplant recipient for the remainder of his or her lifetime. As a predicted outcome of stem cell therapy, being able to be treated without reliance on anti-rejection drugs - both directly after surgery and on a long term basis – would be a much desired improvement in the quality of life for patients.

See (Chamorro, Falcon et al. 2009) for a review of the varied modes of action of these anti-rejection drugs.
The Remarkable Life and Unclear Death of Dolly, the Sheep

Dolly (5 July 1996 – 14 February 2003) was the first mammal cloned from adult cells using nuclear transfer technology. In science, credit often goes to the man who convinces the world of some scientific discovery, not the man to whom the idea first occurs (Darwin 1914). As set out in Supplement 3, the brief history of vertebrate cloning (nuclear transfer experiments) began in the early 1900s. Yet, it wasn't until the birth of Dolly was announced in 1997 (Wilmut, Schnieke et al. 1997) that government and religious leaders throughout the world took note, and began raising their ethical concerns about the possibility that human cloning might soon become a reality.

Sir Ian Wilmut (b. 1944)

Dr. Ian Wilmut, the leader of the research group, named Dolly in response to a suggestion by the individuals who took care of her gestational mother. Dolly Parton was one of their favorite country music singers. If you want to read his 1997 interview see (Ross 1997).

In 1997, Dr. Ian Wilmut of the Roslin Institute in Scotland, head of the team that cloned Dolly, stated that their research was focused solely on animal cloning for a specific purpose other than human cloning. The primary objective of Dr. Wilmut and his team was to develop a new method for a technology called “pharming” in which expensive drugs (such as insulin and tissue plasminogen activator) could be produced inexpensively, in the milk of sheep (see Supplement 3.) (Di Berardino 2001).

Dolly was cloned from the frozen mammary epithelial cells of a white ewe that had died six years earlier. The oocytes used in the cloning of Dolly were obtained from a black sheep. Reports concerning the final years of Dolly the sheep indicated that she was in declining health. On 14 February 2003, Dolly was euthanized because she had a progressive communicable lung disease, a type of cancer common in sheep, and severe arthritis. Sheep of Dolly's breed typically live to be about 11-12 years; Dolly was only 6 years...
old at her death. Roslin's scientists stated that they did not think her lung illness related to Dolly being a clone, and that other sheep in the same flock had died of the same disease. Was there a failure to check all aspects of her medical status before euthanizing her? Was there a failure to report types of possible clone-related health problems? An official autopsy report was never issued about the causes of Dolly's death. It is unclear why more information about Dolly's death was neither examined nor revealed. (See: Schneider 2010).

The landmark paper that described the birth of Dolly inspired scientists all over the world to attempt to use nuclear transfer to clone a variety of animals. While the journal Nature published the cloning of Dolly, the scientists and the editors at Nature recognized the bioethical issues that this technology would elicit. Nonetheless, newspapers all over the world reported this discovery in the framework of cloning. 

SCNT methodology applied to human stem cells

It took about 10 years after Dolly was cloned to apply SCNT to generate human blastocysts. The classical method was first reported by French et al. (French, Adams et al. 2008). Dr. French and his colleagues first obtained 29 oocytes from three young (20–24 year old) women who had previously been egg donors. The recruitment criteria for these women were youth and personal ethical approval of egg donation. The intended women who donated these oocytes provided appropriate informed consent, deemed the oocytes to be in excess of their reproductive needs, and received no financial compensation. All of this ethics-based information was included in their paper. Previously, it was uncommon for published papers to describe how the procedures used in their experiments conformed to bioethical standards. See (Han, Cheng et al. 2010). As described above new methods have been designed to greatly improve the generation of human embryonic stem cells using SCNT.

SCNT method applied to produce human embryos and stem cells

- Oocyte donors, all of whom that had at least one previous successful donation cycle, underwent routine controlled ovarian hyperstimulation typically used in most infertility procedures. The donor cells were primary adult fibroblast cell lines isolated from a 2–3-mm skin biopsy sample obtained from two healthy male volunteers.
- The cumulus matrix was removed from oocytes and the oocytes were enucleated using aspiration.
- A single fibroblast donor cell 10–15 µm in diameter was inserted under the zona pellucida of the enucleated oocyte so that it remained in contact with the cytoplasm.
- The fibroblast nucleus is separated from the cytoplasm of the enucleated oocyte.
- Fusion between the enucleated oocyte and the fibroblast was done in a cell fusion chamber by administration of electrical pulses. These pulses appear to enhance cell fusion.
- Fused cells were then maintained in vitro. Those that proceeded to develop into blastocyes (as measured by morphological criteria) were used to harvest the stem cells from the inner mass
Andrew J. French

Andrew J. French is an animal cloner recruited from Australia by a commercial company called Stemagen. In their experiments on human SCNT, they used skin cells from Dr. Samuel Wood, another Stemagen employee, as the DNA source. In addition, they used 29 eggs donated by young women at the fertility clinic that Dr. Wood managed. Although the embryos grew only to a very early stage, the work could also theoretically be seen as a step toward creating babies that are genetic copies of other people.

It is also interesting that while this paper represented a scientific breakthrough neither the first author, Andrew J. French, nor the last author, Samuel Wood, have published any subsequent paper on Human SCNT since 2008. One reason may be that during that same year, Yamanaka published his famous paper on induced Pluripotent Stem Cells (iPS) (Takahashi and Yamanaka 2006), which does not have the same ethical challenges as SCNT. Moreover, French and Wood worked at a stem cell company. After Yamanaka’s publication of his iPS results, one could speculate that they believed that their further SCNT efforts were no longer economically prudent.

All the experiments by Dr. French were approved by an independent review committee (Anselmo) in accordance with the U.S. Department of Health and Human Services Policy for Protection of Human Research Subjects (45CFR46) and the National Academy of Sciences Guidelines for Human Embryonic Stem Cell Research. All donated oocytes were obtained from three egg donation cycles performed at the Reproductive Sciences Center (RSC), a fertility center located in La Jolla, California, that is fully compliant with the Society for Assisted Reproductive Technologies and the American Society for Reproductive Medicine guidelines.

Mark R. Hughes

Mark Hughes, one the authors on the French et al. (French, Adams et al. 2008) paper, is a pioneer in the field of Pre-implantation Genetic Diagnosis (PGD). His work has centered on understanding gene expression in the early human embryo and the molecular genetics of early embryo development. One of the highlights of his research has been the realization that molecular data from single cells can be used for diagnostic purposes. This led to a multi-year collaboration with IVF clinicians and embryologists at the Hammersmith Hospital in London and the birth of pre-implantation genetic diagnosis as a clinical tool.

Dr. Hughes was one of 11 scientists recruited to start the Human Genome Institute at the National Institutes of Health in the U.S., and has also held the position of Director of the State of Michigan’s “Life Sciences Genomics Hub.” Since 2007, Dr. Hughes has been conducting clinical PGD at the Genesis Genetics Institute in Michigan. This program offers diagnostic services to over 174 human reproductive centers in North/South America and Europe.
Somatic cell nuclear transfer (SCNT) and parthenogenetic embryo development.

A1: **Cumulus matrix-free** human metaphase II oocytes.
A2: Enucleation pipette for aspiration of metaphase plate from human oocytes.
A3: UV fluorescence of human metaphase II oocyte stained with Hoechst 33342.
B1: Pronuclear formation (6–7 hours post-calcium ionophore [CI] activation) in parthenogenetically activated human oocytes.
B2: Embryo cleavage following parthenogenetic activation (day 3).
B3: Parthenogenetically activated (coded reference K1) blastocyst (day 5) from egg donor 3 (coded references F1 and F2).
C1: Pronuclear formation (6–7 hours post-CI activation) following SCNT with an adult fibroblast AF1 (coded references B3 and B4).
C2: Pronuclear formation (6–7 hours post-CI activation) following SCNT with an adult fibroblast AF2 (coded references C5 and C6).
C3: Late day 3 SCNT embryo following nuclear transfer with AF2 (coded references C5 and C6) donor cell.
C4: Early day 3 SCNT embryos following nuclear transfer with AF1 (coded references B3 and B4) donor cells.
C5: Late day 5 SCNT (coded reference 8E) blastocyst following nuclear transfer with AF1 (coded references B3 and B4) donor cell to an oocyte from egg donor 1 (coded references A1 and A2).
C6: Early day 6 SCNT (coded reference K8) blastocyst following nuclear transfer with AF2 (coded references C5 and C6) donor cells to oocytes from egg donor 3 (coded references F1 and F2). Note with attached cleavage arrested blastomere.

Images were captured with a DP70 digital camera attached to an Olympus IX71 reflected fluorescence microscope fitted with relief contrast and differential interference contrast optics (scale bar - 20 m).

Why was Dolly a scientific breakthrough?
For developmental biologists, Dolly's existence challenged several fundamental tenets of their field.

1. Differentiation is a gradual process of specialization that allows the fertilized egg to develop into the hundreds of cell types that make up the entire animal, and was considered to be an irreversible process. Before Dolly was cloned, conventional wisdom by many scientists and the public was that once a cell differentiates, it remains committed to that cell type for the life span of the organism. In differentiated cells, DNA methylation is one of several DNA or chromatin modifications that generally results in gene silencing and, in turn, serves as a critical factor in gene regulation. Consequently, the production of a live lamb from a differentiated mammary cell taken from the mammary gland of a six-year old ewe was a surprising demonstration that differentiated cells are not immutably committed to one cell type. In other words, the genes essential for embryonic development that were silent or methylated in the mammary cells could be turned on again (demethylation) to initiate embryological development. Dr. Wilmut and colleagues' remarkable scientific breakthrough demonstrated that the oocyte has the capacity to reprogram adult cell DNA or to make these silent genes speak, and proved that differentiation was a reversible process. Thus, Dr. Wilmut's study promoted further study in the area of cellular dedifferentiation.

2. The second scientific breakthrough was the fact that healthy gestation and birth was achieved using a nucleus from adult cells that were collected from a sheep that had died and that the cells had been frozen years before the experiment.

3. The third scientific breakthrough that emerged from cloning Dolly was that her telomeres were significantly longer than telomeres obtained from the six-year old sheep. Telomeres, a Greek word for “the part at the end,” are specialized regions at the tip of chromosomes that serve both as a molecular bookend and a cellular clock. Once telomeres are truncated to a specific point, they trigger apoptosis (cell death). Telomeres are quite important in cancer cells where their lengths are not shortened.

4. A final novel and significant aspect of SCNT cloning relates to the origins of DNA replication. In embryonic cells, there are many more sites for replication initiation than are found in somatic cells, which are fully differentiated. The huge size of the nucleus of early embryonic cells is thought to provide a platform or scaffolding necessary to support more places where DNA replications can begin, thereby allowing the DNA to replicate quickly. This is important for rapid cell division and the generation of clones of the embryonic cells. It is unclear how the oocyte can reprogram and rapidly replicate DNA from adult cells used in nuclear transfer technology. A better understanding of these processes will help the basic science and applications of this technology, and to make the process more efficient.

**Telomeres**

Telomeres are located at the end of chromosomes and are composed of DNA repeats associated with
specific proteins. Telomeres can reach a length of 15,000 base pairs and function by preventing chromosomes from losing base pair sequences at their ends. Each time a cell divides, some of the telomere is lost (usually 25-200 base pairs per division). A fetal cell contains long telomeres while cells from an aged individual contain shorter telomeres. In certain aggressive tumors, the telomeres grow longer after each cell division, providing a possible mechanism for how these cells continue to proliferate in an unregulated manner.

Elizabeth Blackburn (b. 1948)
The work of Elizabeth Blackburn and colleagues on telomeres and specifically on telomerase, the enzyme that replenishes the telomere, was acknowledged with the Nobel Prize in Physiology or Medicine in 2009.

Theoretically, one would expect the length of telomeres of the cloned animal to be equal to that of the telomeres found in the donor cell used in nuclear transfer. However, research with cloned calves shows that telomere rebuilding occurs during embryonic development and is critical for successful nuclear transfer.

Thus, Dolly and other cloned mammals expressed rebuilt telomeres during the process of nuclear transfer. In certain cloned animals, telomere length was not only restored but extended beyond that of the donor cell (Wakayama, Shinkai et al. 2000). The implications of these observations may be important in understanding cancer. In many cancerous situations, the telomeres do not shorten and in this way contribute to the continual unregulated proliferation of the tumor cells. Understanding how to better regulate telomere shortening might enable scientists to re-regulate cancer cells.

Thought questions

- Would you clone a beloved pet if it was financially feasible for you?
- Are the Ottos justified in creating a clone that may face serious health risks?
- Is it fair that such technology is available only to those few consumers who can afford it?

(Source: Cohen, Marina Genetic Engineering Crabtree Publishing, 2009)
Cost and medical risks associated with reproductive cloning – Experience with animal models

The following concerns highlight negative aspects of reproductive cloning:

- highly inefficient
- expensive
- associated with significant medical risks.

More than 100 nuclear transfer procedures could be required to produce one viable cloned animal. The dollar cost of cloning a dog or cat is estimated at between $25,000 and $50,000. There are wild estimates that the cost of cloning a human being would be between $1 million and $5 million dollars and would require at least 3-5 years. But aside from the cost and the time involved, human cloning is not an approved procedure. Since there has been no current reported generation of a human clone using nuclear transfer because it is a banned procedure, it has been difficult to assess the medical risks the technology would pose in humans (Lane 2006). We need to extrapolate from the only available data that come from animal studies.

Since there has been no current reported generation of a human clone using nuclear transfer because it is a banned procedure, it has been difficult to assess the medical risks the technology would pose in humans (Lane 2006). We need to extrapolate from the only available data that come from animal studies.

Cloning experiments on mice, for example, reveal some of the health risks of SCNT, such as:

- damage to the immune system
- increased risk of death from pneumonia
- increased development of tumors
- liver failure
- spontaneous abortions
- abnormal births

Notably, out of 12 cloned mice, born-apparently healthy at birth, only two lived out a normal life span of 800 days (Thuan, Kishigami et al. 2010). In cattle, cloning is associated with a high rate of abortion and still births. It is even unclear whether the health problems that Dolly the sheep developed (arthritis prematurely for her breed, as well as a lung disease prevalent in older sheep) were a result of her being kept indoors during her entire life or because she was cloned using SCNT.

In summary, cloned animals exhibit a wide variety of medical abnormalities and defects that depend in part on the specific species being cloned. Nonetheless, of the several commercial companies cloning animals for work or sport (e.g., horses) or for food (e.g., cows, pigs), few report much success (see Supplement 3: Historical Overview of Vertebrate Cloning).
Conclusion

As one can imagine, medical problems observed in cloned animals may be difficult to interpret or to extrapolate to humans for several reasons.

- First, there may be differences in embryological development between humans and animals. Small blebs (protrusions) of plasma membrane-enclosed cytoplasm are associated with early human embryo cleavages. This process is called fragmentation, and is not as common in mouse embryos.
- Second, currently, scientists know far more about human than animal embryo culture, implantation, and post-natal development. In vitro fertilization (IVF) technology is over 25 years old and has yielded tremendous experience in maintaining human embryos in culture. Animal embryo culture and development must be studied far more extensively to know more regarding how and when to relate phenomena and outcomes comparatively to those for humans.
- Third, in animal cloning research, improved screening criteria are needed for selection of the best cloned embryos for implantation. There also is a need for greater understanding of how to arrive at these improved criteria.
- Finally, experiments conducted with animals don’t always translate to humans. Often it is not possible, given our current understanding, to extrapolate directly from animal results to likely human relevance. For all of the reasons stated above, far more needs to be known about animal cloning to derive increasingly accurate and specific results that may – or may not always – shed light on questions in human research.

References


