Update on alternative methods to derive embryonic and embryonic-like stem cells

Strategic delivery:	☒ Setting standards	☐ Increasing and informing choice	☐ Demonstrating efficiency economy and value

Details:	
Meeting	Scientific and Clinical Advances Advisory Committee (SCAAC)
Agenda item	8
Paper number	SCAAC(06/16)03
Meeting date	13 June 2016
Author	Anna Quinn, Scientific Policy Officer
Output:	
For information or decision?	For decision
Recommendation	Members are now asked to: • consider the progress of research (since June 2015) into alternative methods to derive embryonic or embryonic-like stem cells; • advise the Executive if they are aware of any other recent developments; and • reflect on whether their views have changed in light of recent research.
Resource implications	None
Implementation date	None
Communication(s)	Information updates summarised in this paper and SCAAC's view will be used to update the paper 'Alternative methods to derive stem cells' used by the HFEA Licence Committee when considering research licence applications which involve the use of viable embryos for research purposes.
Organisational risk	☒ Low ☐ Medium ☐ High
Annexes	None

1. Introduction

1.1. Human embryonic stem cells (hES cells) have the potential to form every other type of cell in the body. They are important for research into cell biology, drug testing and disease modelling. hES cells could potentially be used in therapies for patients.

1.2. hES cells are derived from cells of human embryos. Currently the only way to derive hES cells involves using viable embryos but researchers are investigating alternative methods of deriving hES cells, or hES-like cells, that do not involve the use of viable embryos.

1.3. Section 3A(1)(c) of Schedule 2 of the HFE Act 1990 (as amended) requires embryo research to be "necessary or desirable" for defined purposes. If alternative methods of deriving hES or hES-like cells are developed, it may not be necessary for research groups to use viable embryos. It is, therefore, important for the Authority to keep up to date with developments regarding these alternative methods so that the HFEA Licence Committee can bear them in mind when considering research licence applications.

1.4. In February 2016, as part of their annual horizon scanning process, SCAAC advised the HFEA that alternative methods to derive hES cells should remain a high priority for the committee and the Authority during 2016/17. This paper summarises key research since June 2015 and is therefore an update to the detailed review provided in SCAAC paper SCAAC (06/15)04.

2. Induced pluripotent stem cells

2.1. One alternative way to derive hES cells is by producing induced pluripotent stem cells (iPS cells). iPS cells are adult somatic cells which have been reprogrammed to an embryo stem cell-like state. This process is controlled by mediators including transcription factors which bind to DNA and alter gene expression, and epigenetic changes which involve changes to the information in the genome over and above that contained in the DNA sequence.

2.2. The following papers summarise the mechanisms of reprogramming to pluripotency, factors which regulate this process and iPS cell protocols:

2.2.1. Nishino & Umezawa (2016) studied DNA methylation dynamics in iPS cells and found that DNA methylation profiles were similar between human iPS cells and human ES cells. Differences in methylation profiles were caused by random aberrant hypermethylation at early passages.

2.2.2. In their recent review, Ji et al (2016) highlight advances in iPS cell generation methods, including the use of patient-specific somatic

cells, and the mechanisms behind reprogramming. They also discuss potential for future development of iPS cells with greater efficiency and safety.

2.2.3. In their 2015 review, Singh et al reflect on the last 10 years of progress in reprogramming. The authors discuss the limitations of iPS cells and current perspectives in the field.

2.2.4. A review by Kumar et al (2015) discusses the use of transposon-based methods to induce pluripotency as opposed to viral transduction of core reprogramming genes. The authors propose that transposon-based reprogramming will advance the establishment of safe, non-viral methods of iPS cell production.

2.3. Potential applications for iPS cells

2.3.1. A recent review by Spitalieri et al (2016) summarises iPS cell methods and clinical applications in the field of monogenic disease modelling and therapy.

2.3.2. Ma et al (2015) carried out a study which showed generation of pluripotent stem cells from patients with mitochondrial disease that contain exclusively wild type mitochondrial DNA (mtDNA). For patients with heteroplasmic mutations, iPS cell lines were generated containing only wild type or mutant mtDNA through spontaneous segregation of heteroplasmic mtDNA in proliferating fibroblasts. Somatic cell nuclear transfer was used to replace mutant mtDNA from homoplasmic fibroblasts. The authors conclude that both reprogramming approaches offer complementary strategies for derivation of pluripotent stem cells containing exclusively wild-type mtDNA.

2.3.3. When considering clinical applications of stem cells it is important to elucidate whether iPS cells can have the same therapeutic outcome as human embryonic stem cells (hES cells). Riera et al (2016) compared human iPS cells to hES cells as treatments for retinal dystrophies in a rat model. Results showed that both pluripotent cell types functioned equally well after being transplanted into the rat subretinal space. This study provide some evidence that human iPS cells could be as beneficial for treating retinal dystrophies as hES cells.

2.4. Issues and possible solutions relating to the use of induced pluripotent stem cells in research, or in clinical therapies:

2.4.1. A recent study by Kang et al (2016) found that human iPS cells had a higher load of mitochondrial DNA mutations compared with skin fibroblasts or blood. They also found that the frequency of mitochondrial DNA mutations in iPS cells increased with age (the age

range of study participants was 24 to 72 years). The authors suggested that these results highlight a need to monitor mitochondrial DNA mutations in iPS cells, especially those generated from older patients, they also suggest examination of the metabolic status of any iPS cells intended for use in therapy.

2.4.2. One potential issue when producing human iPS cells for use in clinical applications is the culture of these cells in media containing xenogenic reagents (cells derived from other species). Wang et al (2015) generated clinical grade human iPS cells using xeno-free culture media in a 'good manufacturing practice' environment. The authors propose that generation of iPS cell lines in these conditions could be valuable in providing cells for future clinical trials and/or therapies.

2.4.3. It has been noted that iPS cells have a higher frequency of epigenetic errors when compared with pluripotent cells obtained using somatic cell nuclear transfer. Tiemann et al 2016 used two different methods of reprogramming to determine whether these epigenetic errors are specific to transcription factor reprogramming in the mouse. Using the first reprogramming method, germline stem cells (GS cells) were converted to pluripotent stem cells using specific culture conditions. Secondly GS cells were converted to iPS cells using transcription factor mediated reprogramming. The study results showed that GS cell-derived iPS cells and germline pluripotent stem cells exhibited similar levels of donor memory and de novo reprogramming errors. The authors therefore conclude that epigenetic aberrations found in iPS cells are not specific to transcription factor-mediated reprogramming.

3. Somatic cell nuclear transfer

3.1. A further method of deriving embryonic stem cells is somatic cell nuclear transfer (SCNT), which is a potentially valuable tool for generating genetically matched stem cells for research and therapeutic purposes. The process involves transferring the nucleus of a somatic cell into an egg cell that has had its nucleus removed. Factors present in the cytoplasm of the egg cell then reprogram the somatic nucleus to pluripotency. The egg cell containing the somatic cell nucleus then develops to form an early stage embryo from which embryonic stem cells can be derived.

3.2. Recent developments in SCNT

3.2.1. Shao et al (2016) investigated the mitotic advantage observed in recipient cells and donor nuclei following SCNT. The results showed that human bromodomain-containing 3 with reprogramming activity (BRD3R) positively regulates mitosis during reprogramming, upregulates a large set of mitotic genes and early stages of

reprogramming and associates with mitotic chromatin. The authors suggest BRD3R as a reprogramming factor and propose that mitosis may be a driving force of reprogramming.

3.2.2. A study by Chung et al (2015) acknowledged that application of SCNT is limited by poor blastocyst formation rate and low efficiency in deriving human ES cells. Here, the authors demonstrate that H3 lysine 9 trimethylation (H3K9me3) is a reprogramming barrier in humans and overexpression of demethylase KDM4A improves human SCNT. This study provides a potential method for improving success of SCNT.

3.2.3. Finally, a recent review by Loi et al (2016) provides an overview of recent developments in SCNT and its potential applications.

4. Naïve human pluripotent stem cells

4.1. In vivo, the pluripotent state emerges during development of the blastocyst. At this stage two initial cell lineages are formed: the inner cell mass (ICM) and the trophectoderm. The ICM is the pluripotent founder population and goes on to form two further lineages: the epiblast lineage and the primitive endoderm. At this point the epiblast cells enter a naïve developmental 'ground state', these cells will go on the form all future cell lineages of the embryo. Until recently, scientists have struggled to derive these naïve human pluripotent stem cells (PS cells). However, in recent years a number of studies have begun to define the conditions required to generate these cells in vitro.

4.2. Recent developments in deriving naïve human pluripotent stem cells

4.2.1. The study by Guo et al (2016) sought to culture naïve human PS cells directly from inner cell mass cells using selective kinase inhibition culture conditions. The authors report that cultured cells express hallmark naïve pluripotency factors and additionally display features of mitochondrial respiration, global gene expression and genome-wide hypomethylation distinct from primed cells. The cells were also shown to transition through primed pluripotency into somatic lineage differentiation. Guo et al. propose that these results support the case for naïve pluripotency in human development that is commonly observed in the mouse.

4.2.2. In another recent study by Qin et al (2016), overexpression of the Hippo pathway effector YAP is shown to promote the generation of naïve PS cells from both human ES cells and iPS cells. It was also shown that Lysophosphatidic acid (LPA) can partially substitute for YAP to generate transgene-free human naïve PS cells. The authors

conclude that their results demonstrate a role for YAP in the human naïve state, with implications for early human embryology.

4.2.3. Zhang et al (2016) carried out a study in mice looking at epigenetic changes and their ability to drive reprogramming of mouse epiblast cells to naïve pluripotency. The authors showed that blocking histone H3K4 methyltransferase MLL1 activity with small-molecule inhibitor MM-401 reprograms mouse epiblast cells to naïve pluripotency. These results confirm that intrinsic epigenetic changes have the ability to drive reprogramming events in vitro.

4.2.4. A study carried out last year by Duggal et al (2015) reported a novel combination of small molecules and growth factors in culture medium that facilitate rapid induction of transgene-free naïve pluripotency in human ES cells as well as in mouse ES cells. The authors propose that in their culture medium, the FGF signalling pathway via PI3K/AKT/mTORC induced the conversion of primed human ES cells towards naïve pluripotency. This study provides evidence of a possible route to naïve pluripotency that does not require ectopic expression of naïve genes.

4.2.5. Finally a review carried out by Wang & Gao (2016) discuss recent studies in human naïve stem cell derivation and DNA methylation analysis.

5. Conclusions

5.1. SCAAC last considered research in this area in June 2015. The committee discussed developments in ground state cells, generation of clinical grade stem cells and SCNT compared with iPS cells. At this time the committee agreed that it was necessary to continue to use human embryos to derive embryonic stem cell lines. This was because there is a need to derive these cells for experimental reasons to test the normality of embryos derived after specific procedures. Further, these cells act as the gold standard to which all other pluripotent cells are compared.

5.2. As in previous years, SCAAC concluded in 2015 that there is still no viable equivalent to embryonic stem cells and therefore the creation of stem cells from embryos may still be considered “necessary or desirable” for defined purposes. The committee agreed to continue to review research in this area on an annual basis.

5.3. Since June 2014 research in this area has continued to evolve with a large volume of literature produced. Scientists continue to address the limitations in clinical application of iPS cells with the ultimate aim to produce clinical grade cells that can be safely used in stem cell therapies. Further research into naïve pluripotency suggests that it is indeed possible to derive human naïve

pluripotent cells similar to those that have previously been derived in the mouse, and these naïve cells have also been successfully derived from iPS cells. In this paper the committee is asked to consider developments in reprogramming methods and to consider the other research highlighted.

6. Recommendations

6.1. Members are asked to:

- consider the progress of research (since June 2015) into alternative methods to derive embryonic or embryonic-like stem cells;
- advise the Executive if they are aware of any other recent developments; and
- reflect on whether their views have changed in the light of recent research.

6.2. Information summarised in this paper and SCAAC's view will be used to update the paper 'Alternative methods to derive stem cells' used by the HFEA Licence Committee when considering research licence applications which involve the use of viable embryos for research purposes.

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