Scientific and Clinical Advances Advisory Committee Paper

Paper title:	**Update on alternative methods to derive ES and ES-like cells**
Paper number:	SCAAC(06/14)01
Meeting date:	4 June 2014
Agenda item:	6
Author:	Anna Rajakumar (Senior Policy Officer)
For information or decision?	Decision
Resource implications:	None
Implementation	None
Communication	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
Recommendation to the Committee:	Members are asked to: • consider the progress of research since June 2013, 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.
Evaluation	None
Annexes	None

1. Lay Summary

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

1.2. hES cells are derived from the 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, without destroying viable embryos. The Committee considers the progress of research in this field annually. This paper highlights developments since June 2013.

2. Introduction

2.1. Section 3A(1)(c) of Schedule 2 of HFE Act 1990 (as amended) requires embryo research to be "necessary or desirable" for defined purposes. If alternative methods of deriving ES or ES-like cells are developed, it may not be necessary for research groups to destroy 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.

2.2. In February 2014, SCAAC advised the HFEA that alternative methods to derive hES cells should remain a high priority for the Committee and the Authority during 2014/15. The Committee also asked to be periodically updated with relevant research developments, and last considered research in June 2013. This paper summarises key research since June 2013 and is therefore an update to SCAAC paper SCAAC(06/13)01.

3. Research

Induced pluripotent stem cells – reprogramming

3.1. Over recent years pluripotent stem cells, derived from different types of somatic cells by nuclear reprogramming, have shown much promise. Since June 2013 further studies have explored the process of reprogramming:

- A study by Chitilian et al, (2014) sought to better understand the mechanism by which p/CIP (a transcriptional co-activator) functions in embryonic stem cell pluripotency. This study drafted a p/CIP gene regulatory network which demonstrates that p/CIP is a component of this extended network and therefore a key factor.

- A new family of pluripotency-related oncogenes, consisting of DPPA2 and DPPA4, has recently been identified (Tung et al, 2013). Findings from this study have important implications for

stem cell biology and tumorigenesis. In order to identify novel pluripotency-related oncogenes, an expression screen for oncogenic foci-inducing genes within a retroviral human embryonic stem cell cDNA library was conducted. The in vitro transformed cells gave rise to tumors in immunodeficient mice.

- A study by Lee et al (2013) looked at the overexpressing miR-302 cluster and observed a significant increase in conversion of partial to fully reprogrammed iPS cells by suppressing MBD2 expression, thereby increasing NANOG expression Therefore, the group suggested, expression of exogenous miR-302 cluster (without miR-367) is efficient in attaining a fully reprogrammed iPS state in partially reprogrammed cells by relieving MBD2-mediated inhibition of NANOG expression. Studies such as this provide a direct molecular mechanism involved in generating complete human iPS cell reprogramming.

- In a study by Luo et al, (2013) the downregulation of the nucleosome remodelling and deacetylation (NuRD) complex was shown to be required for efficient reprogramming. Overexpression of Mbd3, a subunit of NuRD, inhibits induction of iPSCs (induced Pluripotent stem cells) by establishing heterochromatic features and silencing embryonic stem cell-specific marker genes, including Oct4 and Nanog. Depletion of Mbd3, was shown to improve reprogramming efficiency and facilitates the formation of pluripotent stem cells that are capable of generating viable chimeric mice, even in the absence of c-Myc or Sox2. The results of this study establish Mbd3/NuRD as an important epigenetic regulator that restricts the expression of key pluripotency genes, suggesting that drug-induced downregulation of Mbd3/NuRD may be a powerful means to improve the efficiency and fidelity of reprogramming.

- Virally derived human iPSC (hiPSCs) have been compared with human Embryonic Stem Cells (hESC) using episomal non-integrating vectors (Polanco et al, 2013). The study reported that the virally derived hiPSCs exhibit a propensity to revert to a pluripotent phenotype following differentiation. This instability was revealed using a published method to identify pluripotent cells undergoing very early-stage differentiation in standard hESC cultures, by fluorescence activated cell sorting (FACS) based on expression of the cell surface markers TG30 (CD9) and GCTM-2. Cell populations from hESC and episomally derived human iPSC did not show these abnormalities.

Amniotic stem cells

3.2. Many different cell types have been found within amniotic fluid with much potential for pluripotent cells isolated from this source. It has been suggested that amniotic epithelial (AE) cells in particular possess ES- and iPS cell-like pluripotent differentiation characteristics (De Sacco et al,

2010). Amnion cells also have additional advantages of being retrieved in a non-invasive way and can be frozen and stored easily. Recent studies have suggested that stem cells derived from this source have increasing potential to maintain genetic stability and possess pluripotent characteristics and therefore this source has been monitored as part of our annual review. Therefore the studies below have been highlighted:

- Recent research conducted by Corradetti et al, (2013) aimed to obtain and characterise, for the first time in bovine species, presumptive Mesenchymal stem cells (MSCs) from the epithelial portion of the amnion (AECs) and from the Amniotic Fluid (AF-MSCs) to be used for clinical applications. AF-MSCs and AECs are positive for the pluripotent markers (POU5F1 (OCT4) and MYC (c-Myc)) and lack of the hematopoietic markers. When appropriately induced, the study found both cell lines are capable of differentiating into ectodermal and mesodermal lineages.

- Hartmann et al, (2014) looked at amniotic fluid derived stem cells giving rise to neuron-like cells without a further differentiation potential into retina-like cells. Their data suggests that amniotic fluid derived cells are an interesting cell source, which may also give rise to neural-like cells. However, a more specific differentiation into neuronal and glial cells could not be shown conclusively, and the author highlights that further investigation would be required to explore this in more detail.

Somatic Cell Nuclear Transfer (SCNT)

3.3. Finally it is important to note that, as in previous years, there have been some recent developments in research exploring the potential to derive human embryonic stem (hES) cells from somatic cell nuclear transfer. This technique uses skin cells, which are taken from an adult. The nuclear genome from this cell is then placed inside an oocyte, stripped of its own DNA. Until recently researchers have struggled to encourage the oocyte to divide (meiotic arrest takes place inducing a rapid exit from the metaphase stage) and reach blastocyst stage. Further to the research of Tachibana et al, (2013), highlighted in SCAAC's discussion in June 2013 (SCAAC 06/13)01), the following research has recently been conducted:

- Moulavi et al, (2013) explored the effect of technical steps of SCNT on different aspects of cloned embryo development in sheep. In vitro-matured oocytes were enucleated in the presence or absence of zona and reconstituted using three different SCNT techniques: conventional zona-intact (ZI-NT), standard zona-free (ZF-NT) and intracytoplasmic nuclear injection (ICI-NT). The results of this study indicate that technical aspects of cloning may result in the variety of cloning phenotypes.

3.4. Making clinical grade stem cell lines without destroying an embryo

- Rodin et al, (2013) isolated a human recombinant LN-521 isoform and developed a cell culture matrix containing LN-521 and E-cadherin, which both localise to stem cell niches in vivo. This allows clonal derivation, clonal survival and long-term self-renewal of hES cells under completely chemically defined and xeno-free conditions without the use of Rho kinase inhibitors. LN-521 and E-cadherin alone did not enable clonal survival of hES cells. The study suggests that the matrix developed allows for hES cell line derivation from blastocyst inner cell mass and single blastomere cells without having to destroy the embryo. The group purports that this method can facilitate the generation of hES cell lines for development of different cell types for regenerative medicine purposes.

4. Conclusions

4.1. When SCAAC last considered the progress of research in June 2013, the Committee was interested in new developments in iPS cells and in SCNT ES cells. The Committee discussed specific research by Tachibana et al, (2013) which showed for the first time that ES cells can be derived from embryos generated from adult cell nuclei reprogrammed via SCNT into oocytes. This provides the opportunity to compare iPS and ES cells from an individual. The group suggested that SCNT ES cells could potentially provide a more clinically relevant source of cells, however further work is required to assess both iPS and SCNT ES cell safety and therapeutic potential.

4.2. The Committee agreed that ES cells are still the "gold standard" and, while iPS cells are extremely useful for studying diseases, their variability and genetic instability (increased chance of carrying mutations and uncertain epigenetic status) is higher due to the way they are derived. This may make them unsuitable for clinical use. Further research needs to be conducted exploring this instability and potential epigenetic effects.

4.3. As in previous years, SCAAC concluded in 2013 that, despite promising developments in the iPS cell creation process and the potential to derive stem cells from other sources, there was 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.

4.4. In this paper the Committee is asked to consider developments in the reprogramming methods used to create iPS cells and to consider the other research highlighted, such as work using amniotic stem cells. The Committee should also carefully consider the progress recently made in developing the process of somatic cell nuclear transfer to produce ES cells.

5. Recommendations

5.1. Members are now asked to:

- consider the progress of research (since June 2013) 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.

5.2. 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.

6. References

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