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Acknowledgements
We thank B. Fell, C. Blackstone and C. Smith for valuable discussions. We regret that we could not cite all the relevant literature owing to space constraints.

Competing interests statement
The authors declare no competing financial interests.

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ESSAY — DEVELOPMENTAL CELL BIOLOGY

Ethical sourcing of human embryonic stem cells — rational solutions?

Martin Evans

Abstract | At the heart of the extensive ethical and regulatory debates that have surrounded human embryonic stem cells is the human pre-implantation embryo. Advances in the understanding of cellular reprogramming, both by cell nuclear replacement and by potential new protocols, should lead to methods that circumvent the use of a practicably viable embryo.

There has been considerable anxiety, ethical debate, and regulatory and legislative intervention on both national and international scale in response to the advent of cell-culture growth of human embryonic stem (ES) cells and the far-reaching ideas for their application in future cell-based therapeutic interventions (BOX 1).

Although undoubtedly appropriate and necessary, much of this debate has far outrun both the present and potential scientific and medical realities. Moreover, some of it is based on misconception and misinterpretation. Here, I outline a series of hypothetical examples, each coupled with predictions for scientific and medical possibilities, which might lead towards more rational and considered approaches to the regulation of the derivation and use of human ES cells.

Ethical debate and the rush to regulate

Human cells have been isolated, propagated, studied and used in cell culture for many years. The first cell line, HeLa, was established in 1952 (REF. 1). Tissue and organ transplantation, employing both autologous and heterologous transplants, are important, well-established tools of modern therapeutic practice[2]. Transplantation therapies using both primary and tissue-culture cells also have a long history[3].

Why then, when human ES cells were derived, and when their use as a source of cells for cellular transplantation therapies was suggested, was there so much ethical debate and a rush to regulate? There are two reasons. First, these cells are derived from human pre-implantation embryos, and second, the technology has been confused with that of reproductive intervention.

Isolation from viable embryos. In most cases, human ES cell lines have been derived from a culture of a pre-implantation embryo produced by *in vitro* fertilization (IVF)[4,5]. These have mostly been 'spare' embryos in excess of those required for reproduction and donated by couples who have undergone IVF treatment. These embryos — as evidenced by the successful development of their siblings — probably had the potential to develop into children. It is this developmental potentiality that has marked them out as different from other cellular donations that lies at the core of the ethical sensitivities.

Cells for transplantation therapies, as well as for *in vitro* studies, can be isolated from aborted fetuses. (In the UK, research in fetal tissue is currently governed by the Polkinghorne Committee Report, 1989 (REF. 6.)) Embryonic germ cell lines (that is, pluripotent stem-cell cultures that are closely related to ES cell lines) can be isolated from 5–9-week-old fetal gonads[7]. Here, putting aside for a moment the ethical dilemmas of the termination of pregnancy, there was no potentiality for development at the time when the cells were isolated.

Confounded by reproductive technology. The debates that surround the derivation and the use of human ES cells have been confounded by concepts of reproductive embryo-manipulation technologies and genetic engineering. Manipulation of embryos in culture has provided a powerful experimental technique in both laboratory and farm animals. In the mouse, ES cells have provided an important tool for genetic manipulation. In addition, one possibility for producing histocompatible cells for the treatment of patients would be to generate specific embryos using nuclear transplantation of adult-cell nuclei into oocytes[8] (see below).

Appropriate regulation is needed

Both the ethical derivation and the appropriate use of ES cells, and their associated technologies, need to be subject to suitable regulation. Because medical technologies are global and transcend frontiers, these regulations would preferably have an agreed international ethical and legal framework. The ethical issues, however, arise directly from present technologies, and regulations conceived now should neither impede useful progress nor fail to forbid abuses or excesses. Here, I comment on the likely and desirable future scientific and technical developments that I think might eventually render much of the present regulatory angst unnecessary.

Box 1 | **What is a stem cell?**

The body consists of a large number of specialized, differentiated cells with diverse functions that are organized into specific tissues and organs. During development, and throughout life, many of these tissues can repair and regenerate themselves after tissue damage.

This regeneration and repair depend on populations of less-differentiated cells — stem cells. They are quiescent but can enter into rapid division when required and undergo specific differentiation. The essential property that defines a stem cell population is that, by division, these cells can both maintain the reserve pool and provide precursor cells that can develop into the final differentiated state. Some stem cells have limited potential, such as the epidermal stem cells of the skin, which give rise to keratinocytes. Others, such as bone-marrow haematopoietic stem cells, can develop into a wide range of blood cells.

These stem cell populations are specialized in (that is, committed to) specific directions of differentiation. So, it has been surprising that some studies have shown that stem cells extracted from one tissue have repopulated another. However, in most cases, these studies were not carried out with single purified cell populations, and these results are by no means unambiguous[26]. Nevertheless, highly specialized, tissue-specific stem cells are exactly what is needed for a particular therapy if they could be isolated in sufficient numbers.

It has also been possible to isolate stem cells that have a wide range of differentiative potential, because these provide the stem cell population in the very early embryo. Such embryonic stem (ES) cells are pluripotent — that is, they are still at the base of the differentative tree and have retained their embryonic capacity to give rise to most, if not all, cell types. The potential use for ES cells is their ability to be isolated and grown in large numbers, coupled with their ability to differentiate into any other cell of the body. This might provide a way to generate populations of specifically pre-determined somatic stem cells and precursor cells, which will allow the therapeutic regeneration of damaged adult tissues for which there is no other endogenous or sufficient source.

The therapeutic opportunities that can be foreseen to arise from human ES cells all depend on cell and tissue transplantation. Transplant therapy is well established in medical practice and provides life-saving treatments — ranging from blood transfusion to heart, lung and liver transplantation. Apart from the surgical challenges and those of infection transfer, all the main problems arise from the incompatibility of donor and host tissues. Where a patient can provide his or her own donor cells or tissue, for example, in skin grafting or with stored autologous bone marrow, there is a perfect tissue match and no problems of immune rejection.

A hypothetical example. Let us consider the following scenario. A person suffers a heart attack, and it would be possible to repair and strengthen the damaged heart muscle by implanting new heart muscle cells. This procedure, whereby heart muscle cells derived from ES cells are transplanted back successfully into a damaged heart, has already been shown to work in animal experiments[9]. We are, however, a long way from a practical therapy, but the principles are being established[10]. We therefore need a source of heart muscle cells that matches the patient perfectly. If this were a skin graft, we could find a suitable patch of skin somewhere on the patient to donate the necessary cells. However, the only heart muscle cells reside in the damaged heart and do not regenerate sufficiently. (Contrary to previous dogma, there is now mounting evidence that there are cardiomyogenic stem cells in the adult heart[11], see REF. 12 for a review.) Can we find a new source of heart muscle cells for this patient that would provide a perfect match?

This patient's body contains the required components. If the nucleus of a mature egg cell from one of her ovaries is replaced by one from another cell in her body, an embryo may be formed, and from this an ES cell culture could be established. This is the technique used by Hwang *et al.*[13] who took nuclei from cumulus cells and transferred these into their associated oocytes, thereby producing embryos that are genetically identical to the female donor. From one of these embryos, they reported the successful isolation of an ES cell line. ES cells might readily differentiate into immature heart muscle cells and these could be used for the transplantation to cure the heart condition of the patient. No other individual has been involved in providing tissue for transplantation. So, how could there be any ethical barrier to this procedure?

"One possible solution would be to have a stem cell bank of pre-prepared ES cell lines from which a tissue match might be found for most patients."

The problem lies with the embryo. Although never allowed to develop beyond a few dozen cells, the embryo had the theoretical potential to develop (in a host uterus) into a human being. This would, however, have been reproductive cloning — a procedure that is already illegal in many countries. It is important to point out that in animal experiments it remains a very inefficient process, and many of the resulting fetuses are abnormal. If not legally (let alone practically) possible, why should this theoretical abuse stand in the way of otherwise perfectly acceptable therapeutic benefit?

The problem remains with the embryo. The above process of cell nuclear replacement (CNR) — which is equivalent to the first step of reproductive cloning — leads to an embryo. But also notice that, as this embryo is genetically identical to the patient, no new, genetically distinct individual has been formed. Until now, human ES cells have all been derived by putting early embryos into tissue culture. These have mainly been 'spare' embryos donated by couples undergoing IVF treatment and have not been made by CNR. They are, therefore, not only potential humans, but also pointedly often the siblings of IVF babies. They are the result of fertilization and therefore represent genetically new individuals. So, although the spectre of cloning, in particular of reproductive cloning, has inspired much of the public concern and legislative regulation, the CNR procedure, when viewed purely as a process for reprogramming an adult's cell nucleus so that it might form an ES cell, is seen to sidestep an important ethical hurdle.

Extension of the scenario. In the scenario above, the female patient provided her own source of ES cells, which would be a perfect genetic match. A close approximation to such a perfect match could be obtained using a donor-derived egg cell and a nucleus from one of the patient's own cells — in this case, the only genetic components that could cause a potential mismatch reside in the mitochondria of the egg. Because this is an identical cellular manipulation, we might be assured of the same moral and ethical considerations. The only remaining questions concern the donation of the oocyte. It is important to keep in mind that this oocyte is an unfertilized egg — a cell from the adult person, not a cell from an as yet unborn embryo.

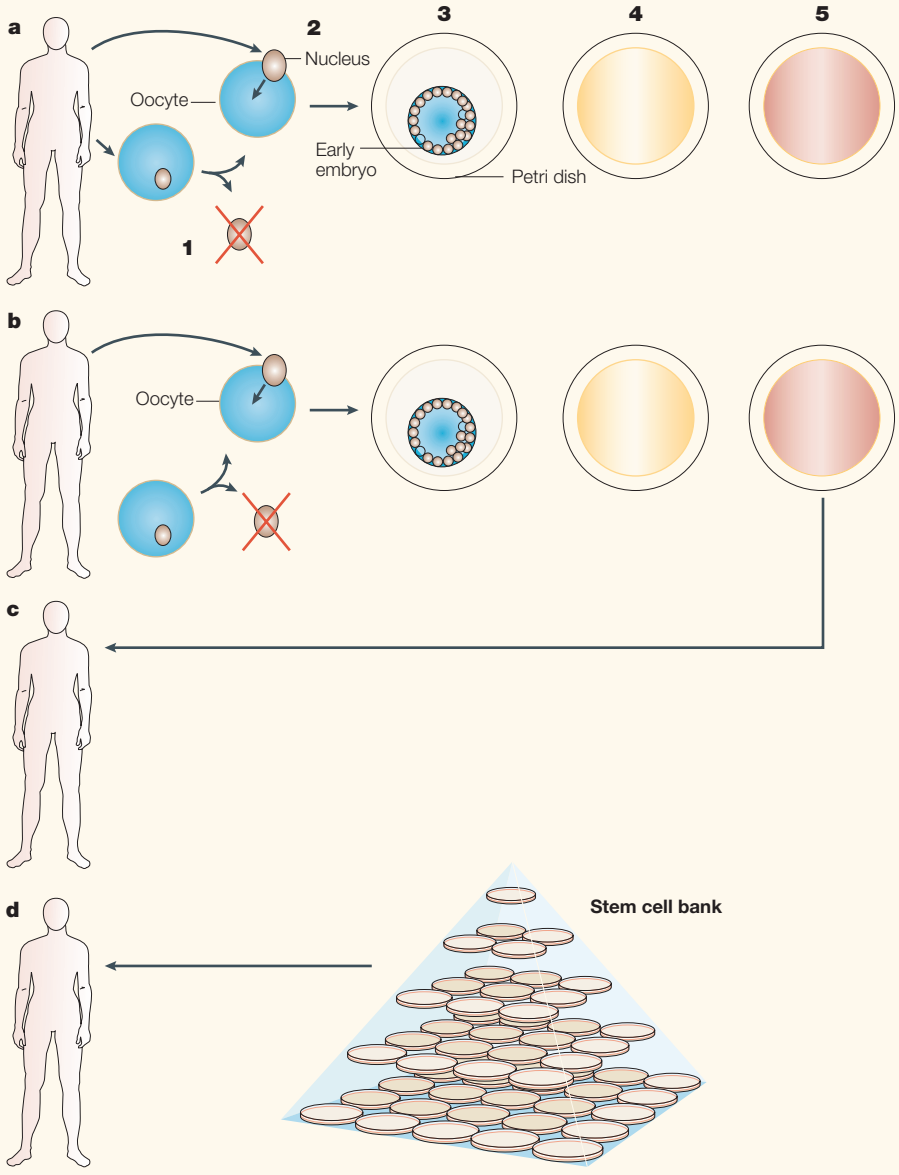

Figure 1 | **Different scenarios for cell nuclear replacement procedures.** The diagram summarizes the different ways (**a**–**d**) of sourcing histocompatible, committed precursor cell populations for cellular therapy by derivation of embryonic stem (ES) cells from embryos produced by cell nuclear replacement (CNR). **a** | The patient provides both the somatic nucleus and the oocyte. **b** | The patient provides the somatic nucleus, but not the oocyte. **c** | A patient can use a cell line originally produced by and for another patient. **d** | Patients can be treated by selection of a suitable cell line from a stem cell bank created by CNR. The CNR procedure involves the following steps: the oocyte nucleus is removed and discarded (step 1); a nucleus from an adult cell is transferred to the oocyte (this is the actual process of CNR) (step 2); an early embryo results (step 3) and is immediately used to make a culture of ES cells, which can be maintained and proliferated, and large numbers of identical cells can be stored (step 4). Finally, as required, some of the ES cells are used to differentiate into specific tissue precursor populations ready for therapeutic use (step 5).

Stem cell banks

The CNR-based recycling of a nucleus from a differentiated adult cell back to an ES cell might (perhaps surprisingly) be a more ethically acceptable route than using spare IVF embryos. However, there are practical problems to consider. The supply of donor oocytes might be limited, the efficiency of this approach could be low, and the whole procedure could be costly and time-consuming when a rapid therapy might be needed. One possible solution would be to have a stem cell bank of pre-prepared ES cell lines from which a tissue match might be found for most patients. This bank would have to be extensive to provide perfect matches for most patients, perhaps with tens of thousands of pre-made and pre-characterized cell lines.

Bradley *et al.*[14] have recently provided a useful, detailed analysis and have come to the conclusion that as few as 250 cell lines would be able to provide a reasonable, but imperfect, degree of matching for many patients. The matching would be equivalent to what is now often accepted for kidney transplants, but would provide relatively few patients with a perfect match. Much more stringent matching is required for transplants that will regenerate components of the immune system.

This scenario might seem the best commercial option compared with the *ad hominem* production of every cell line for every patient. But the tissue matches would be imperfect for many patients, and sourcing the cell lines with full ethical permissions for indefinite future use could be problematic. Given the considerations mentioned above, it might be ethically preferable to generate these lines by a CNR-based procedure rather than from IVF embryos, and obtain the appropriate informed consents and permissions. To create a bank with the maximum useful coverage for tissue histocompatibility, it would be particularly useful to be able to preselect donor cell genotypes for the establishment of ES cell cultures. By using the CNR route, such preselection of desirable histocompatibility haplotypes would allow a greater degree of coverage from a smaller number of ES cell lines. Bradley *et al.*[14] point out that the donor genotypes that are most useful are those that are homozygous at the appropriate loci and therefore carry the minimum number of different transplantation antigens. These could be sourced from adult donors. FIGURE 1 summarizes the different CNR scenarios.

Regulating reproductive technologies

Human ES cells are potentially equivalent to mouse ES cells, which have been widely used as a vehicle for genetic manipulation. They have the potential, in combination with a normal embryo, to form part (or all) of the resulting adult mouse. This mouse is derived from cells that were originally grown in tissue culture and into which specific new mutations may have been introduced. These new mutations may then be transmitted into the mouse population by direct breeding. This could provide a pathway for genetic manipulation of the human germ line, which raises issues of eugenic misapplication and abuse — and this obviously needs to be controlled.

"…it should never be the existence of human ES cells or their use in the laboratory as cell cultures that we should seek to control, but their reproductive misuse…"

An explicit legal ban on reproductive cloning allows laboratory CNR procedures to proceed with confidence. Similarly, secure regulation against the genetic misuse of ES cells would allow greater confidence in their therapeutic application. However, it is not appropriate to seek to control the existence of human ES cells — only to control their application. I would argue that it should never be the existence of human ES cells or their use in the laboratory as cell cultures that we should seek to control, but their reproductive misuse when the ES cell is in itself only one potential component of misapplication. In addition, ES cells provide only one of several different routes for genetic misapplication.

Regulation of medical use

The ethical and moral considerations regarding human cells can be split into, first, aspects relating to their origin and permissions for that origin and, second, the uses and applications of these cells.

If a patient's own cells or tissues are used for medical therapy, the ethical considerations would seem to depend entirely on the medical ethics of the procedure, including the informed consent of the patient. When transplant tissue from another individual is used, the interest of the patient lies entirely in the suitability of the transplant for the medical procedure being undertaken. There is a risk–benefit calculation, which encompasses the likelihood of successful outcome and the likelihood of adverse complications, including tissue rejection and adventitious infection. The other major consideration is whether the donor, the donor's representatives, or society might be harmed by the donation. Society might also be harmed by the use of the procedure itself, and the individual good could be in conflict with societal good. Considerations of societal harm might in fact be at the core of much of the ethical unease concerning human ES cells.

It has been shown in mice that a lymphocyte can provide the donor nucleus to make a mouse embryo after transplantation into an oocyte[15]. Does this mean that a blood sample has to be accorded the same potentiality as an ES cell? Conversely, perhaps it would be better to confer on a human ES cell culture no greater or lesser respect or ethical status than a blood sample.

There seem to be two basic arguments for the special status of human ES cell lines — their origin from an embryo and their potential to develop again within an embryo. Once again, the basis of the ethical problem is the embryo. I have argued that the potentiality is shared, by virtue of the technology, with many other cell types and, therefore, the potential of a cell to provide genetic material for future embryo development cannot be an absolute criterion. The problem remains that ES cell lines are produced by explantation of an embryo and, therefore, the destruction of that embryo's own future as a fetus. I have suggested that CNR, by producing a cloned embryo that has no legal or moral future, does partially mitigate this problem. In the future, ES cell lines could perhaps be produced without involving an embryo.

Can we get around the embryo?

Almost all of the moral and ethical dilemmas stem from, and centre around, the fact that ES cells are derived from a potentially viable embryo. There have been suggestions to circumvent this by using embryos that lack full viability — for example, parthenogenetically derived embryos[16] (these do not usually survive to term in experimental animals) or, alternatively, by inserting a lethal transgene at the time of nuclear transfer. These ideas, especially the latter, are technical fixes for regulatory hurdles[17], which should not be necessary and are at best unfortunate, if not specifically deleterious. If, however, the process from an 'adult' committed cell to an 'embryonic' pluripotential cell did not involve a viable implantable embryo, the ethical dilemmas must fall away. Human ES cells are typically isolated by tissue culture from an early developing human embryo that is not yet implanted into a uterus and that contains only ~100 cells (a blastocyst), although mouse experiments indicate that slightly later stages might also be used. However, there is one report of the isolation of mouse ES cells directly from isolated cleavage embryo blastomeres[18]. Even here, there is a transient cleavage-stage embryo. The question remains, should the product of an oocyte dividing immediately in tissue culture be considered an embryo?

"…it is important to remember that it is not ES cells themselves that are needed for therapy, but the derived, committed precursor populations."

Recently, experiments in mice have shown that stem cells with properties that are apparently equivalent to ES cells might be isolated from a newborn mouse testis. Kanatsu-Shinohara *et al.*[19] have shown that, using culture conditions that are conducive to ES cell growth, these cells can routinely be established in culture and that they have many of the properties of mouse ES cells. This is interesting because germline stem cells remained active in the human testis throughout most of the adult life. Perhaps a testis biopsy could provide a simple and non-contentious source of pluripotential human cells?

Direct reprogramming

Could there be another way around the 'embryo issue'? The differentiated state of a cell is the result of a series of developmental steps, each of which typically narrows the prospective fate of the cell and its descendants. This is, in most cases, an irreversible restriction, although there are some well-documented examples of transdifferentiation. We know, however, that the genetic constitution of most vertebrate differentiated cells remains unchanged despite their restricted fate. This was most convincingly shown by the nuclear transplantation experiments in amphibians in which some, and eventually all, development was supported by a nucleus from a differentiated cell, which was transplanted into an enucleated oocyte[20] (for a comprehensive review, see REF. 21). Subsequently, with the advent of Dolly the sheep[22], this dramatic demonstration of the oocyte's ability to reprogramme the nuclei of adult differentiated cells has been extended to mammals and now repeated in a number of species. We know that the differentiated state of an adult cell nucleus can be reversed. This metastable state of differentiation is set up by nucleo-cytoplasmic interactions and is maintained by transcription-factor networks and secondary modifications of chromatin and of DNA, all of which are reversible. It should be possible to dissect the process of reprogramming seen in CNR in an oocyte and possibly devise

methods to reprogramme a cell's nucleus without an intervening step that involves a viable embryo.

Interestingly, it has been known since Miller and Ruddle's experiments almost 30 years ago[23] that pluripotent embryonic cells can reprogramme the nuclei of differentiated cells in the context of cell hybridization. This result has been confirmed repeatedly and been shown to be applicable to ES cells, as well as embryonal carcinoma cells, and to be consistent across species. The process of reprogramming is now being dissected, and Do and Scholer[24] have shown that the factors that are necessary to initiate reprogramming reside in the nucleus, but not in the cytoplasm, of ES cells. It has also been shown that *Xenopus laevis* oocyte cytoplasm is capable of initiating reprogramming of mammalian nuclei or isolated chromatin, which allows the mechanism to be closely studied[25].

If these developments come to fruition, and pluripotent cells can be generated *in vitro* without the requirement for an embryo, these cells might be equivalent to ES cells and might have all of the potential advantages of ES cells as a basis for a wide range of cellular therapeutics, but should merit none of the present restrictions.

Concluding remarks

The explosion of ideas for new, cell-based therapeutic opportunities all depends on an ability to manipulate the developmental and differentiation potential of cells. ES cells are providing us with a starting material to develop this ability. But their derivation depends on an ability to dedifferentiate adult-derived cells. The differentiated state is a metastable condition, which is the result of transcription-factor feedback loops and secondary modifications of DNA and chromatin. All of these factors are reversible, but as yet we know only one proven route from an adult cell to an ES cell — that is through CNR and an embryo. Moreover, it is important to remember that it is not ES cells themselves that are needed for therapy, but the derived, committed precursor populations. When we have mastered methods for manipulation of the differentiated state of cells, there should be alternative routes for deriving the desired patient-specific therapeutic cell populations.

Martin Evans is at the Cardiff School of Biosciences, Cardiff University, Cardiff CF10 3US, UK.
e-mail: evansmj@cardiff.ac.uk

doi:10.1038/nrm1698
Published online 15 July 2005

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Competing interests statement
The author declares no competing financial interests.