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PROSPECTS

Are There Morally Acceptable Alternatives to Blastocyst Derived ESC?

Mahendra S. Rao*

Department of Neuroscience, School of Medicine, Johns Hopkins University, Baltimore, Maryland 21205

Abstract ESC derivation, use and SCNT have raised many moral and ethical issues. In this opinion piece I have focused on the argument that morally less ambiguous alternatives to ESC derived from the ICM of blastocysts exist. These possibilities range from using multiple adult stem cell populations each of which is uniquely suited for a particular disease target or identifying adult ESC-like populations, using transdifferentiated ESC-like cells or alternate methods of deriving ESC. I suggest that while it is important to support such efforts current results do not provide sufficient compelling data to allow one to stop the use of ESC and perhaps adult cells will never be a reliable alternative. All options need to be fully explored and decisions need to be made with scientific rigor and respect for each individuals moral compass. J. Cell. Biochem. 98: 1054–1061, 2006.

Key words: hESC; karyotype; characterization; mitochondrial sequencing; methylation

Pluripotent primate embryonic stem (hES) cells, isolated from the inner cell mass of preinplantation embryos, are able to self-renew and to generate any cell type of the developing embryo [Thomson et al., 1995, 1998]. Because of their remarkable ability to proliferate and differentiate, hES cells are potentially useful for both in vitro developmental studies and for invivo cell replacement therapies. Current reports comparing basic cell properties of pre August 9th lines have suggested that human ES cells are overall similar in their expression of cell surface antigens and markers characteristic of the ES cell state [Ginis et al., 2003, 2004]. While not all markers have been tested in all lines expression of oct3/4, rex-1, utf, connexin, gap junction proteins, SSEA, and TRA antigens is common as is the ability to self-renew and differentiate into ectoderm, endoderm, and mesoderm in vitro and in vivo. This common set of properties is relatively unique to the ESC stem cell population and serves to distinguish blastocyst derived plurpotent stem cell lines from other stem cell populations derived at different stages of development. Hallmarks of ESC are summarized in Figure 1.

ESC however are a recent addition to the known populations of stem cells and are neither the best funded or the closest to clinical applications. Hematopoitic stem cells were identified more than 30 years ago and cord blood cells, neural stem cells, mesenchymal stem cells, limbal stem cells, etc. have all been transplanted in patients who have shown therapeutic improvement or in many cases complete recovery. The NIH funding pattern acknowledges this difference with a roughly 10:1 bias of funding towards adult stem cells.

DO ESC HOLD A SPECIAL PROMISE?

There is in general no dispute among scientists of the utility of adult stem cells and their potential therapeutic role. Indeed the only stem cells or stem cell containing products in clinical use are those derived from adult populations. Bone marrow transplants, limbal stem cell therapy, cord blood transplants, pancreatic islet

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Mahendra S. Rao's present address is Invitrogen Corporation, 16000 Faraday Road, Carlsbad, CA 92008.

*Correspondence to: Mahendra S. Rao, Gerontology Research Center; Stem Cell Biology Unit/Laboratory of Neurosciences; National Institute on Aging, National Institutes of Health, Baltimore, MD 21224.
E-mail: raomah@grc.nia.nih.gov

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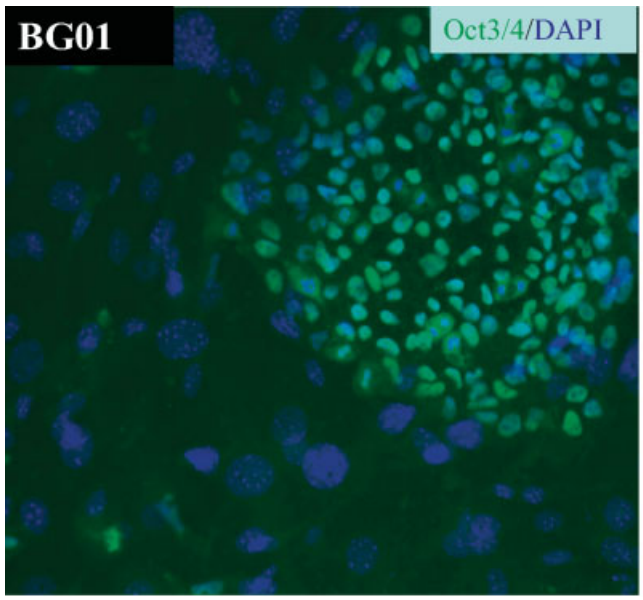

ESC properties

Open transcriptome

Unique methylation profile

Absence of senesence, high TERT activity

Lack of growth inhibition

Ability to generate germ cells and from teratomas in nude mice

Presence of characteristic set of markers

Absence of markers of differentiation

Fig. 1. ESC are a unique population. A typical colony of a human ESC line with cells stained with Oct3/4 and Hioechst dye to identify all nuclei is shown and the table to the right lists the properties that distinguish ESC from all other stem cell populations.

transplants, carticell (a cartilage progenitor product) are all examples of successful adult stem or progenitor cell therapy. In contrast, while ESC from mice have been used successfully to derive transgenics and chimeras, and we have known about mouse ESC since the 1980's no ESC or ESC product is currently used in the clinic.

However, many scientists who work on adult stem cells have extended their efforts to study human ESC as well. There are several reasons for this in my mind. One simple reason is obtaining sufficient numbers of cells for therapeutic use. While hematopoietic and mesenchymal stem cells can be obtained in relatively large numbers from adult tissue, the same cannot be said for other adult stem cell populations. Skin, liver, gut, skeletal and cardiac muscle, neural tissue are all tissues where the presence of stem cells has been demonstrated but in these same tissues obtaining sufficient numbers of cells for clinical use has proven extremely difficult. This difficulty has been technical in the sense that it has been difficult to propagate and amplify adult stem cells in culture. Mesenchymal stem cells can be propagated for about 5–100 passages other cells for fewer and in general no most cells cannot be passaged more than fibroblasts before undergoing senescence or undergoing transformation. Indeed, despite considerable effort over the past 25 years it has been difficult to passage HSC for even 3–5 passages without losing their engraftment or long-term repopulation ability. This has been true for cord blood cells, neural, and other stem cell populations despite occasional reports to the contrary.

This is in contrast to ESC which appear to escape senescence and can be propagated relatively indefinitely (reviewed in Reference Rao [2004]). Cells can be passaged, stored and repropagated, and truly large numbers of cells can be obtained. This ability to grow is maintained in vivo and small numbers of cultured and propagated ESC when transplanted into a blastocyst can generate an entire animal. This singular ability to grow for much longer periods of time in culture than any other stem cell population distinguishes ESC from all other stem cells and given their ability to differentiate into all major phenotypes makes them a uniquely valuable cell. There are several other reasons to study ESC derivatives including identifying mechanisms to derive and maintain adult stem cells, studying developmental mechanisms, or developing a source for adult populations for which no adult source is available. However, as indicated earlier perhaps the single most important reason is the idea that a bank of cells can be generated that can be used to generate an appropriate cell type when necessary.

ARE THERE ADULT CELL EQUIVALENTS OF HUMAN ESC?

An important argument made by several scientists is that while it is true that ESC have unique self-renewing properties, it is unnecessary to use ESC as ESC-like cells can be isolated from morally less reprehensible sources (Fig. 2). Alternatively multiple adult stem populations each of which is uniquely suited for a particular disease or tissue can be used as alternatives to ESC's. Further, opponents have argued that while adult stem cells are not ESC nevertheless they can be reprogrammed to behave as if they are pluripotent. All three arguments have been well developed by opponents of ESC research who suggest that even if the taint on using ESC is considered small surely if an alternative existed that it would be morally better if one used such an alternative source of useful cells. The logic is quite reasonable and has justified to some extent a funding ratio of 10:1 (in terms of NIH grants) in favor of adult stem cells.

CAN WE HARVEST ENOUGH ADULT STEM CELLS FROM EACH TISSUE TO ELIMINATE THE NEED FOR ESC?

A strong argument made by opponents of ESC cell research has been why work on ESC when adult stem cell populations from various tissues exist? Opponents point to the identification of stem cells from the heart, skeletal muscle, brain, and other tissues where stem cells were not known to exist. They also point to evidence that at least some stem cell populations can be harvested from cadaveric tissue [Palmer et al., 2001; Roisen et al., 2001] and that the current progress suggests that it is only a matter of time before we will have adult stem cells from all tissues that are good candidates for stem cell therapy. In addition, proponents suggest that even if equal numbers of cells were available from ESC derived or adult sources adult cells would be better, as they would be better suited for the adult environment given they existed in that environment.

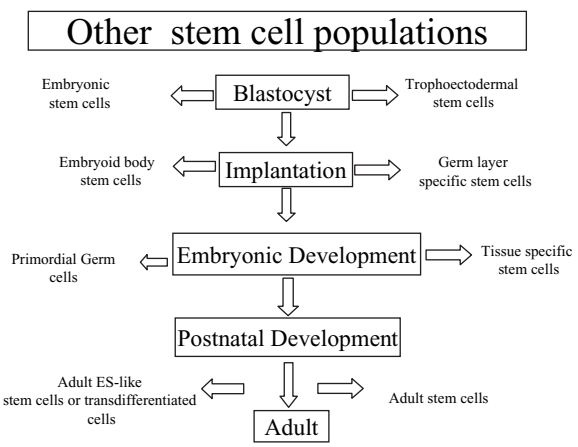

Fig. 2. Stem cell populations. As development proceeds stem cell populations with different properties can be isolated. Pluripotent populations are listed on the left and more restricted stem cell populations are listed on the right. Note that pluripotent cells can potentially be harvested from multiple stages of development.

These are reasonable arguments and I for one have no quarrel with them except to the extent that this is not true for many tissues that are strong candidates for stem cell therapy. Parkinson's disease is one example. While adult neural stem cells have been described by a variety of investigators to date no one has succeeded in generating sufficient numbers of authentic dopaminergic neurons from adult stem cells. Even in the few reports that have shown some dopaminergic differentiation, for example, the numbers are several orders of magnitude less than that from fetal tissue. Investigators have concluded that it would take at least three fetal tissue donations to treat one side of the brain in a Parkinsons disease patient and therefore even that is not a sufficient source. One can extend this to argument to many other tissues including the heart and skeletal muscle where to stem cells can be harvested and propagated to a limited extent.

I think it is fair to say that adult stem cells when abundant are a strong alternative to ESC cells and indeed are the preferable choice. I, for example, would prefer autologous bone marrow to ESC derived HSC if autologous bone marrow was available. However, if an abundant source was unavailable then I would certainly hope that I could use ESC derived HSC. I note that this is not an uncommon situation even in the HSC field. Cord blood has been used as an alternative when marrow derived HSC are unavailable and cord blood researchers readily acknowledge that a single cord blood sample is generally insufficient for an adult patient and would love to have additional sources of cells.

On a practical level for researchers this means going with whatever is the best source of cells and not excluding any potential cell source, as clearly no single source is sufficient.

No ESC proponent can reasonably argue that ESC sources render adult sources unnecessary and likewise no adult stem cell proponent can reasonably argue that adult stem cells at the current state of knowledge are a viable alternative to ESC and therefore research into ESC can be stopped (Fig. 3).

ARE ADULT PLURIPOTENT STEM CELLS A VIABLE OPTION?

Several types of pluripotent stem cells have been described (Fig. 3). Pluripotent stem cells that are similar to ESC but derived from later stages of embryonic development such as epiblast cells, EBD cells, and germ cells [Shamblott et al., 2001; Lakshmanan et al., 2005]. Persistent pluripotent cells [Young et al., 2004a,b] that have been described as existing in some tissues by several (albeit small number of investigators). These include umbilical matrix derived cells, MAPC's, PESSCC, and other ES-like cells [Jiang et al., 2002; Keene et al., 2003; Mitchell et al., 2003]. In general ESC have been defined by a rigorous criteria that includes cell-cycle regulation, marker expression, ability to generate germ cells, contribute to multiple lineages, and generate teratomas in nude mice. Most reported adult pluipotent stem cell populations do not fulfill all the criteria. Adult pluripotent cells do not appear to express ES cell markers, do not form teratomas, and if injected into blastocysts do not go germ line with any fidelity and their contribution to various germ layers if shown has been unimpressive (Fig. 4). Until and unless such cells have the properties reported and the results are independently documented one would argue that one cannot stop work on ESC based on their existence.

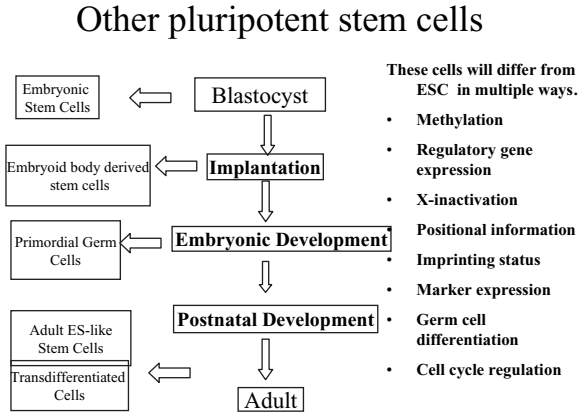

Fig. 3. Pluripotent stem cell populations. Pluripotent stem cells as defined by the ability to contribute to ectoderm, endoderm, and mesoderm have been isolated by multiple investigators at multiple stages of development although it is unclear if they share overall similarity in critical parameters that will define their overall ability to function appropriately in vivo.

IS TRANSDIFFERENTIATION OF ADULT STEM CELLS A SOLUTION?

A second class of pluripotent stem cells has also been described these are cells that are normally nor pluripotent, but can be derived from adult cell populations including adult stem cells by a process of dedifferentiation or transdifferentiation (Fig. 5). Such processes have been described by numerous investigators (see review [Liu and Rao, 2003]). Though this field is by no means absent controversy. Alternate explanations of some of the observations labeled as transdifferentiation highlight the importance of critically reviewing the evidence, testing for contaminating populations of cells, performing clonal and FISH analysis, and carefully assessing the robustness of the phenomenon. It appears that many (though by no means all) of the plethora of reports documenting transdifferentiation can be explained by overenthusiasm in interpretation, presence of contaminating crest/vent, or circulating HSC's or by cell fusion. In the past we have argued that transdifferentiation is possible, we know something about the process and even have some indications of small molecules that could regulate this process. However, this does not suggest to us that transdifferentiated cells are clinically relevant as yet. Unless we have precise control of transdifferentiation, we cannot apply this idea to clinical treatment. Further since this process may involve a cell conversion not typically found in normal development for the most part it is unclear how reliable and reproducible it will be in vivo. These concerns suggest that while transdifferentiated cells may serve as a substitute for ESC cells in the future one cannot at this juncture offer a strong argument that ESC work should be stopped, as a sufficiently reliable alternative existed.

IS IT LIKELY THAT ADULT STEM CELLS CAN TRULY BE ESC LIKE?

While I have argued that current results demonstrating adults stem cells can serve as a source of ESC-like cells leave much to be

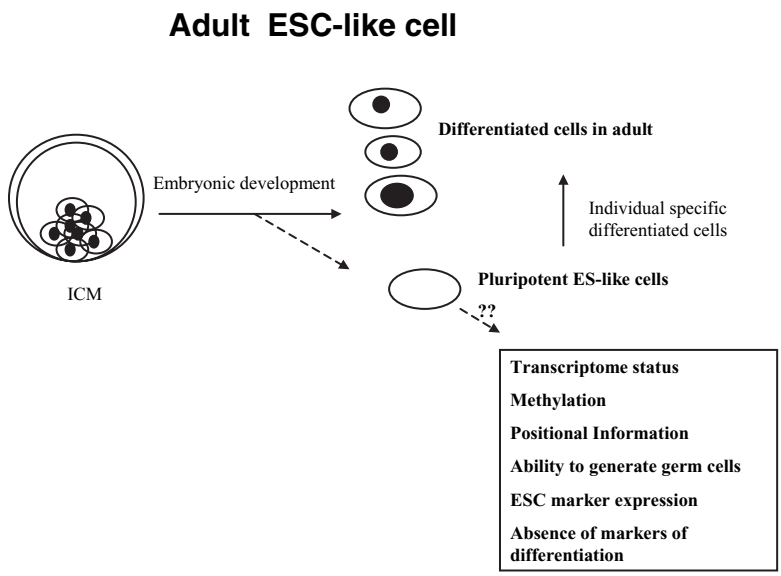

Fig. 4. Pluripotent stem cells in the adult. Several pluripotent stem cell populations have been isolated from adult tissues and data suggests that such cells may represent an alternative to ESC. However, critical differences may nevertheless exist that may make such cells less attractive for clinical and basic biology use.

desired, this does not necessarily imply that this will always be true. Perhaps technology for transdifferentiation will change, the data on adult persistent pluripotent cells will be validated or perhaps sufficient numbers of stem cells from all targeted tissues will be identified rendering the requirement of embryonic stem cells moot. While it is difficult to predict the future one can perhaps examine carefully what makes ESC unique and what changes occur when embryos develop and ask if those changes may be perhaps more challenging barriers than one had assumed.

As the organism develops several genetic and epigenetic changes occur. These include x chromosome inactivation in females, acquisition of cell-cycle regulation by p53 and rb, allele specific gene expression of approximately 10% of the genome, loss of immortality, change from an open transcriptome to permanent shutting down of large regions of the genome in a cell and tissue specific fashion, acquisition of positional

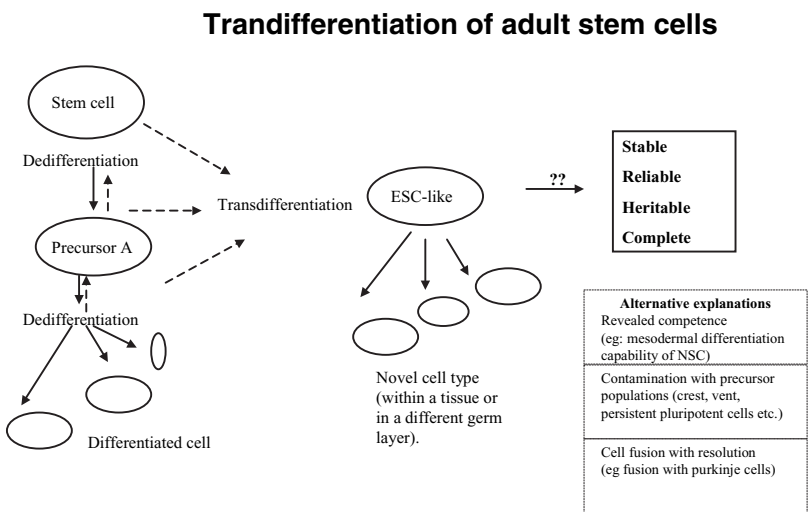

Fig. 5. Trandifferentiated cells. The process of transdifferentiation has been documented extensively and is illustrated. However, several problems remain that need to be carefully evaluated.

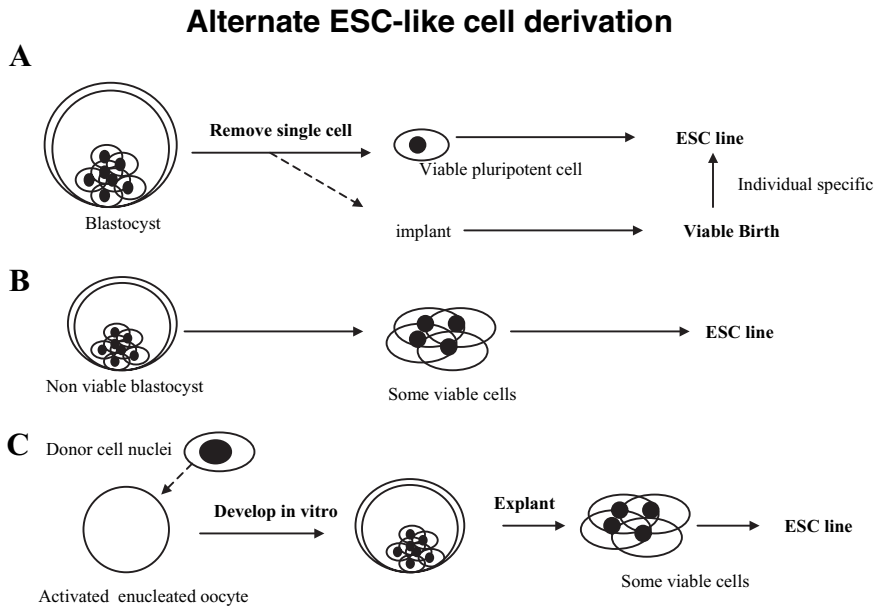

Fig. 6. Alternate methods of deriving ESC. Three potential methods of deriving ESC are shown in panels **A**, **B**, and **C**. These do not represent the only potential methods but these illustrate the potential, less morally ambiguous methods, that are being discussed.

information, etc. These changes appear to be an intrinsic part of the developmental program and small alterations in the process are detrimental to optimal development. These changes occur in all cells including stem cells and are the basis of the difference between adult and embryonic stem cell populations. For adult stem cells to revert to and ESC state either because some persisted into the adult or one induced trans-differentiation one would have to reverse this process rather precisely as even small changes cause disease. Current data identifies the technical challenges we face but our understanding of the processes is limited and does not suggest that any breakthrough simplifying process is on the horizon. It does indicate to us however that it is unlikely that with current technology we will be able to generate ESC-like cells anytime soon or that this process even when it will be developed will be technologically straightforward.

Paradoxically the only way to reprogram nuclei has been to use blastocysts or fertilized eggs (hence the small but consistent success of somatic nuclear transfer and adult cell reprogramming).

More recent studies have suggested that this reprogramming factor is a cytoplasmic component of ESC and fertilized eggs, and as such would require developing techniques to test, purify, and identify this factor with the associated requirement of additional embryo's, embryonic cell lines, or oocytes.

Such studies however are currently ineligible for research funding to a large extent and as is true for ESC cells being isolated without destroying the embryo (see below), current rules may not allow us to develop alternatives that might satisfy the ethical concerns of a larger group of people than the current number of ESC supporters.

CAN ESC BE DERIVED WITHOUT DESTROYING THE EMBRYO?

Another suggestion put forth by some ethicists has been that perhaps one can bypass all ethical concerns by generating ESC without destroying the embryo (Fig. 6). One can imagine a process of removing a single cell from a developing balstocyst (as is done for genetic diagnosis) and using this cell to derive an ESC. Others have suggested that just like organs can be irreparably damaged so can the blastocyst. However, individual cells within these blastocysts are viable and could be used to derive ESC lines that lack any moral issues. Defining death at this stage while difficult could be done using logic similar to that used in defining death in other difficult situations. Others have noted

that it is possible to take an unfertilized egg and activate it so that it begins to mature as if fertilized and derive parthenogenetic lines. One can imagine taking this a step further by transferring somatic cells nuclei into an unfertilized egg and then activating it to get ESC. Further one can imagine working with germ cell lines which are derived from later stage embryos that not subject to the same rules as blastocysts. Germ cells in rodents have been shown to be pluripotent, capable of contributing to chimeras, and generating entire progeny. Germ cell lines have been identified from human tissue as well though they do not appear to be as easy to propagate or have the same degree of self-renewal as ESC. Germ cells nevertheless remain a viable alternative.

Each of these methods carries its own set of issues and requires technical skills that are either available only in a restricted number of centers or require technology that needs to be developed. These techniques may in the future perhaps allow one to avoid the ethical dilemma that some face in using discarded blastocysts to generate ESC. I would note, however, that developing the expertise requires working with blastocysts, deriving lines for experimental purposes and performing experiments that are currently against the law. Paradoxically perhaps current rules may not allow us to develop alternatives that might satisfy the ethical concerns of a larger group of people than the current number of ESC supporters.

SUMMARY

Studying ESC and deriving additional lines, and SCNT and the possibility of generating human clones and/or chimeras has raised many moral and ethical issues. There is no consensus on these issues internationally or even within the United States. Groups have been quite polarized in their views and there appears some confusion in the debates. I would suggest that this is not one single issue but several issues each of one must be evaluated by ones own moral compass that weigh risk, cost, justice, failure to provide available aid, religious, and other issues. In the absence of consensus among different religions one must respect the views of all and weigh the choices available as fairly as one can. Above all this is a dynamic field in which science may alter the context of the debate and may render some issues moot. Currently I would suggest that for the narrow argument that morally less ambiguous sources of stem exist at present is one that is difficult to sustain based on current knowledge.

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