SUBMISSION

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As a scientist with extensive experience in the maintenance and differentiation of mouse ES cells, and with hope that this work might eventually find use for human therapy, I would like to comment on the issue of ES cell-based therapies in three specific areas.

1. Embryonic Stem (ES) cells vs Adult Stem Cells:

Exploitation of adult stem cells for cell and gene therapies has received enormous attention. Several clear deficiencies retard progress in this area:

1. **Breadth of application:** The technology is only applicable to tissues and cells that renew as a consequence of stem cell differentiation. This precludes application to a multitude of clinical conditions.

2. **Identification and isolation:** In general, we are not advanced in the identification or culture of adult stem cells for most biological systems. Indeed the evidence for their existence is often tenuous and based on transplantation experiments. Robust stem cell cultures of the quality (especially purity) required for expansion and genetic manipulation are not commonly available.

3. **Proliferation:** Even where adult stem cells have been defined rigorously and can be identified in vivo and in vitro, there have generally been considerable difficulties in maintaining these cells in an undifferentiated state in vitro, and in achieving long term and efficient proliferation. It is therefore difficult to grow sufficient cells for therapeutic transplantation.

4. **Genetic manipulation:** In the absence of proliferation, effective genetic manipulation of adult stem cells cannot be achieved. Cells that do not divide are not easily infected with retroviruses. Further, cells that do not divide clonally are refractory to alteration of endogenous genes by homologous recombination. These deficiencies have proven to be a major barrier to gene therapy. Further, existing technologies for genetic manipulation of adult stem cells such as random DNA insertion or viral infection carry with them inefficiencies and additional dangers (ie unexpected mutation) which severely limit clinical application of the techniques.

By contrast, ES cells, at least in the mouse, possess key features that appear to make them ideally suited for therapeutic use in cell therapy:

5. **Breadth of application:** ES cells are demonstrably pluripotent and can therefore give rise to all cell types. While precise selection and differentiation protocols await definition, the application of ES cell therapy should not be restricted, allowing treatment of currently untreatable disease.

6. **Proliferation:** ES cells are immortal in vitro. It is therefore relatively simple to proliferate ES cells to the numbers required for effective transplantation.

7. **Genetic manipulation:** ES cells proliferate clonally in vitro. This allows modification of endogenous genes by homologous recombination, a much more effective methodology for achieving genetic cure or novel function than by the addition of new DNA or viruses.

8. **Suitability for transplantation:** The differentiated or partially differentiated cells produced from ES cells appear relatively 'embryonic' in phenotype and behaviour. This suggests that they will retain the developmental plasticity associated with the more primitive state which will assist with integration into tissue following transplantation.

Exploitation of these attributes is anticipated to give rise to therapies for human cellular and genetic diseases that are untreatable or treated only at great expense such as Parkinsons Disease, Gauscher's Disease, stroke, Huntingdon's Disease, dystrophies, macular degeneration, organ transplantation etc.

Research into ES cell therapies is of particular interest in Australia because we have significant local expertise in what may become a new form of medicine, and an industry of enormous value. Within the country we have researchers in Canberra associated with the first report of primate ES cells, researchers in Melbourne who are leaders in the areas of ES cell maintenance and ES cell isolation, and researchers in Adelaide with key expertise in the differentiation of ES cells. Australian biotechnology companies with interests in this area have already been established.

2. Cell deprogramming and reprogramming.

ES cell differentiation: Our own work shows that ES cell differentiation will be achievable in controlled and homogeneous fashion. Importantly, we are now able to differentiate ES cells as a population, through the formation of specific germlayers and into cells likely to be of therapeutic use. The differentiated cultures do not appear to be contaminated with residual stem cells, providing confidence that tumours such as teratocarcinomas will not result from transplantation of the cells.

Cell deprogramming – relevance to ES cell-based technologies: Wordwide, interest in cell 'deprogramming' is accumulating at a remarkable rate. Publications already show 'dedifferentiation' of adult CNS stem cells into neural lineages in rat neonates, differentiation of bone marrow to hepatic lineages and skeletal muscle following transplantation, and differentiation of cells derived from skeletal muscle to haemopoietic cells in vivo. The initial results therefore support the contention that microenvironments within the mammal retain signals that can direct the fate of transplanted cells to a locally appropriate outcome. Together with the generation of partially differentiated embryonic equivalents from ES cells (see 8 above) this provides considerable hope for the therapeutic relevance of cells derived by ES cell differentiation.

Deprogramming and generation of ES cells: Our own work (Rathjen et al., 1999, Lake et al., 2000) shows that the earliest ES cell differentiation events are fully reversible in culture. The generality of this observation is unknown, but it extends to primordial germ cell lineages. There is therefore some reason to believe that pluripotent cells might ultimately be attained by direct dedifferentiation of somatic cells. This would provide a route to generation of ES cells in the absence of embryonic intervention.

Similar possibilities arise from exploitation of nuclear transplantation. Validation of this technology by the creation of Dolly indicates the possibility for generation of ES cells by dedifferentiation following nuclear transfer. While this might be ethically controversial if carried out by transfer of nuclei to oocytes, many scientists are hopeful that it will prove possible to revert a somatic nucleus to a more primitive, pluripotent state by intercellular nuclear transfer. This would occur in the absence of oocyte injection and creation of a viable embryo.

3. ES cells and Embryos

Embryonic stem cells are demonstrably pluripotent but do not appear totipotent, lacking the capacity to form extraembryonic lineages resulting from trophectoderm diffeentiation. This is important because in the absence of extraembryonic lineages, embryogenesis cannot occur. This is consistent with the fact that transplantation of ES cells to an ectopic site does not result in formation of an embryo, but rather a teratocarcinoma.

Given the success of nuclear transfer, it appears that the ES cell is no more potent to generate life than other somatic cells. Each would require reprogramming of the nucleus to an earlier embryonic state followed by transplantation into a uterus in order for life to emerge.