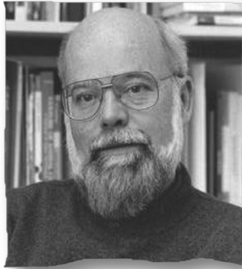
Article

Induced pluripotent stem cells: how to deal with the developmental potential



Hans-Werner Denker is Professor Emeritus at the University Duisburg-Essen, Germany. His main research interests are the cell biology of early mammalian development, embryo implantation, and stem cells, with a recent focus on related ethical aspects. He studied medicine and zoology at Hamburg and Marburg, completing his doctorate under Friedrich Seidel, a pioneer of mammalian developmental biology. Until 2006 he held positions at the Max-Planck-Institut für Immunobiologie, Freiburg, the RWTH Aachen and the University Duisburg-Essen (Germany) as Chair of Anatomy and Developmental Biology. He has published many research papers and some books. He is Editor-in-Chief of the journal *Cells Tissues Organs* (Karger, Basel).

Dr Hans-Werner Denker

Hans-Werner Denker
Institut fuer Anatomie, Universitaetsklinikum, Universitaet Duisburg-Essen, Hufelandstr. 55, D-45122 Essen, Germany
Correspondence: e-mail: hans-werner.denker@uni-due.de

Abstract

Recent developments in research on embryonic stem cells and induced pluripotent stem cells suggest that potentiality of cells should be a new focus in stem cell research ethics and policy. Successful reconstitution of viable embryos from induced pluripotent stem cells using tetraploid complementation has been reported and indicates a way for direct cloning of individuals from these cells. This together with recent observations on gastrulation and pattern formation processes in cultures of embryonic stem cells has considerable ethical relevance after the advent and worldwide spread of induced pluripotent stem cell technology. Available knowledge of the molecular basis of mammalian embryology now makes it possible to envisage ways to deal technically with the ethical dilemma of stem cell potentiality.

Keywords: *developmental potential, embryonic stem cells, ethics, induced pluripotent stem cells, pluripotency*

Introduction

Induced pluripotent stem cells (iPSC) are receiving considerable interest because they can be obtained without any need to sacrifice embryos, while views about ethical questions connected with embryo destruction in the course of derivation of embryonic stem cells (ESC) are still seen controversially in different countries (Holm, 2004; Steinbock, 2007). Cells from which iPSC can be derived (e.g. fibroblasts) are easy to obtain and are ethically non-problematic. Also, iPSC can have exactly the genotype of a prospective transplant recipient (thus avoiding 'therapeutic cloning' by nuclear transfer to an oocyte) at least if recent attempts are successful to replace the induction of genetic modifications (e.g. by retroviral transduction) by epigenetic means and the choice of appropriate cell types (Shi *et al*., 2008; Kim *et al*., 2009). Therefore, it may appear desirable and ethically sound to make iPSC even more readily available, for research and eventually for transplantation purposes, than ESC.

In this sense it might appear consequent that one element of a recent widely publicized initiative (Personal Genome Project, 2008) is to produce iPSC on a large scale and make them openly available internationally. However, ethical implications connected with the potentiality of these cells seem to have largely escaped public attention. This may be due to the fact that most reports have focused on the main intention of this group (Personal Genome Project, 2008), i.e. to sequence the genome of a large number (an envisaged 100,000) of volunteers and to make these data available together with the health records of the same individuals. What has largely escaped attention, however, is that these volunteers are being asked at the same time to also agree to produce iPSC from fibroblasts which they have to provide, and that these iPSC are to be made freely available internationally with the same logic as applied to the DNA data (Personal Genome Project, 2008; Singer, 2008). This, however, needs to be contemplated critically and must be considered very problematical for ethical reasons and gives us an example for recent developments that ask for a re-thinking of stem cell policy.

Viable individuals can be cloned from iPSC (in the same way as from ESC) by the direct cloning procedure of tetraploid

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complementation (TC), a method that does not require using oocytes. TC is an approach that differs considerably from nuclear transfer insofar as it does not involve creating a new ‘synthetic’ cell. Instead, in this case, an embryo is created from ESC (or iPSC) by transferring them to a peculiar microenvironment, i.e. by combining them with tetraploidized helper cells of blastomere type, or blastocysts (for an illustration and a discussion, see Denker, 2006). It has already been shown that TC does indeed work well with iPSC in the mouse (Wernig *et al*., 2007). Experts have no doubt that TC would also be possible in the human, although so far there are no reports in the literature on any cloning by TC in the human. Remarkably, however, it has been proposed recently to introduce cloning of human embryos by TC into IVF–embryo transfer programmes in order to increase success rates (Devolder and Ward, 2007). The proposed protocol would include: generation of ESC from IVF embryos, ESC expansion and storage, generation of a number of genetically identical embryos by TC, possibly storage of surplus embryos, and transfer. Those authors propose to generate the tetraploidized helper cells of trophoblast type from the same ESC, although it still has to be shown whether this is feasible in the human (Devolder and Ward, 2007). The present agreement dominating the western world, that cloning of human individuals should remain banned, may indeed not hold for long: the logic behind such proposals (TC in IVF–embryo transfer) could persuade people to accept cloning of embryos (in fact, of a theoretically unlimited number of genetically identical embryos) as an acceptable means for increasing IVF–embryo transfer success rates, which would obviously imply a deviation from present attitudes, although, in this case, cloning is meant to be used as an intermediate step in the procedure and not the end-point (it is not the aim to produce a number of genetically identical newborns). Apart from this, however, we cannot be sure anyway that reproductive cloning will remain banned in all countries because, for example, some Buddhist authorities would ban embryo destruction (e.g. in the course of therapeutic cloning) but not at all reproductive cloning (depending on the time of ensoulment they are envisaging; Schlieter, 2004).

Also illuminating is to take a side view to aspects of patentability, specifically to the fact that the potentiality of stem cells can be an obstacle to patenting: the availability of the TC technology, no matter whether already in use in human medicine or not right now, forces us to consider pluripotent cells (ESC and iPSC) as potential human individuals, not just an individual DNA but clones of individual human life. Cells as units of life are systems that possess a degree of autonomy anyway. This characteristic, however, gains a considerably higher ethical relevance if their developmental potential is such that organismic wholeness can emerge when TC is performed. TC shows us clearly in case of ESC and iPSC that we are dealing with a very peculiar type of cells which, following the same logic as applied for early embryonic cells (blastomeres), have to be regarded as non-patentable (Denker, 2008a). Although patenting is not intended as part of the mentioned initiative (Personal Genome Project, 2008), the planned wide and largely uncontrolled distribution of the cells touches upon aspects of individual integrity and dignity. It seems that the initiators of that cell donation programme as well as commentators have not seen these implications so far.

Discussion

The peculiar potentiality of iPSC and ESC should be regarded, in philosophical terms, as more close to an active than to a passive potentiality. It is a characteristic of these cells that they tend to autonomously create complexity by initiating pattern formation processes (self-organization in embryological terms) whenever they start cell differentiation, even *in vitro* or after transplantation to ectopic sites (teratoma formation). This phenomenon of morphogenesis, shown impressively in embryoid bodies (discussed by Denker, 2004; Aleckovic and Simón, 2008), is biologically similar to major processes going on during embryonic development, which, in cascades of events, lead to what is called ‘emergent properties’ connected with increasingly higher levels of complexity (Gilbert and Sarkar, 2000). The inherent drive to show such a behaviour is in sharp contrast to the classical example usually given in philosophy for ‘passive potentiality’, i.e. the property owning to a block of marble such that this may be converted into a beautiful statue by a skilled artist.

In the biological literature, there has recently been a wave of publications about gastrulation events in ESC cultures (Gadue *et al*., 2006; ten Berge *et al*., 2008; Nakanishi *et al*., 2009). Although the primary interest of researchers is nourished by the desire to understand and manipulate the differentiation of the germ layers (and their derivative cell types) and so focuses on cell types but not necessarily on the development of spatial order (pattern formation), recent data suggest that the degree of order attained during ESC ‘gastrulation’ *in vitro* is/can be much higher than most people previously assumed, and consequently self-organization and axis formation phenomena in embryoid bodies have now become a hot topic for ongoing research (ten Berge *et al*., 2008). While the focus of that research is on elucidating the involved gene activation cascades, e.g. Wnt and bone morphogenic protein signalling events, it is remarkable that new data show increasingly that the events which are observed in these cultures of pluripotent cells seem to mirror in an astonishing way what is going on during embryogenesis. This leaves its traces in the literature insofar as authors of ESC papers are using more and more often the terminology of embryology when describing embryoid body differentiation, and increasingly do so even without questioning this, i.e. they talk about gastrulation and primitive streak formation, and even about the formation of an anterior–posterior axis (anterior, middle and posterior primitive streak) *in vitro* (Gadue *et al*., 2006; ten Berge *et al*., 2008; Nakanishi *et al*., 2009). The degree of order that can develop in primitive streak equivalents *in vitro* was considered remarkable and astonishing by the authors of one of these recent papers (ten Berge *et al*., 2008). Retrospectively, the observation by Thomson *et al*. (1996) about the formation of impressively well-structured embryonic anlagen with a ‘primitive streak’ in dense cultures of marmoset monkey ESC now does not appear so exceptional or even unthinkable anymore (as it was considered to be by some commentators, at least during the ESC ethics debate in Germany; Beier, 2002). However, from the developmental biology point of view, there are reasons why such pattern formation potential can indeed be expected to be present in ESC (Denker, 2004). With respect to iPSC, it must be added that all we know now about their biological properties is that these appear to be practically identical with those of ESC, including

the behaviour in biological test systems, teratoma formation and TC. Thus, although little data have been published so far on pattern formation abilities of iPSC in embryoid bodies, we must also assume that these should be very similar to what is observed with ESC. Specifically, gastrulation capacity must be present in iPSC, as we can extrapolate from their germ layer formation capacity (teratoma) and basic body plan production ability (as seen after TC: Wernig *et al*., 2007; in this case, pattern formation capacity is of course aided by the helper cells).

Why are these facts about the early embryonic pattern formation potential of pluripotent cells ethically relevant? Formation of the primitive streak is not only the hallmark of, but it is also instrumental in, individuation: this is not only the structure where the definitive endoderm and mesoderm are formed (via gastrulation, involving the process of epithelial–mesenchymal transition and associated inductive events) but it is also instrumental in the formation of the main body axes, specifically the anterior–posterior axis, in a complex process in which the so-called organizer (located at the anterior end of the primitive streak, i.e. at the node) plays a central role (for a review, see Denker, 2004). The critical phase of basic body plan formation and individuation ends when the primitive streak is fully formed, and it is only up to this time point that twinning can occur. This is the reason for defining day 14 of development as a limit for embryo experimentation in British legislation. Gastrulation in the primitive streak, germ layer formation and basic body plan formation/individuation are now being studied extensively in the mammalian system and much insight into genes and signalling systems involved has been gained in recent years (Tam and Loebel, 2007). It has become possible now to envisage specific genes and cellular processes that one can focus on when wanting to make sure that the cells that one is putting into culture do not have the potential to initiate such a process of early embryonic pattern formation/individuation. During the process of iPSC derivation, gene expression patterns are being modified anyway. At present the focus is on gain of function of 'pluripotency genes' like *Oct-3/4*, *Klf4* or *Sox2*. I suggest that an additional and new focus should now be on loss of function (or on avoiding gain of function) of gastrulation/individuation-related genes and signalling molecules. The logic behind this would be to make sure the increased potentiality that cells acquire as a result of their genetic/epigenetic modification does not include the gain of individuation potential. In order to find candidate genes and signalling factors, one just needs to take a look into appropriate papers of the types mentioned, on gastrulation during embryogenesis or on the differentiation of embryoid bodies *in vitro* (Tam and Loebel, 2007; ten Berge *et al*., 2008).

Such an approach would be somewhat related to proposals to repress (permanently or temporarily) the expression of the *Cdx2* gene in the course of production of ESC, the so-called altered nuclear transfer concept (Hurlbut, 2005). The *Cdx2* gene is essential for the differentiation of trophoblast, an extra-embryonic tissue involved in embryo implantation in the uterus (and possibly also in axis-relevant signalling; Denker, 2004) so that Cdx2 mutant embryos cannot implant and so are not viable. However, it can be argued that knocking out (or temporarily knocking down) such a gene just creates severely handicapped embryos (or corresponding pluripotent cells) that theoretically could be saved (Holm, 2008). Specifically it does not necessarily make sure they cannot gastrulate and thus start a process of individuation in the sense just discussed, if appropriate conditions are provided. Thus instead, I propose to envisage gastrulation/individuation-related genes and signalling systems. As discussed, this should now indeed appear feasible (Denker, 2008b). When contemplating the ethical implications of such a strategy it should be seen that the setting is different in case of iPSC as compared with ESC: knocking out/down of genes in the course of ESC derivation can be seen as a procedure of actively disabling cells that are (or would normally be destined to become) totipotent, although alternatively they could be saved (Holm, 2008). In the case of iPSC, the originally non-pluripotent cells need to be manipulated anyway during derivation, and while the main point in reprogramming is the acquisition of new potential, the question is how wide a potential this should be.

What seems to be needed is a change of research focus. The goal should not be just to create what is now usually called 'pluripotent' cells. It seems that a property inherently attached to this 'pluripotent' state is early embryonic pattern formation potential (gastrulation/individuation potential). As experimental data suggest, there seem to be certain links between germ layer formation and pattern formation potential (as embryology anyway suggests), not only in totipotent/pluripotent cells in real life, i.e. in the embryo, but also in 'pluripotent' stem cells (ESC and iPSC) (Denker, 2004). However, for cells to express a defined differentiation potential, it is not necessarily required to also possess early embryonic pattern formation (gastrulation, individuation) potential. If we want to avoid the ethical dilemma presented by the morphogenetic potential of ESC and iPSC, we should change the predominant research focus used in creating stem cells. What appears desirable is to create well-proliferating stem cells with a restricted pattern formation potential, i.e. excluding early embryonic pattern (basic body plan) formation. When testing the properties of the created cells we should not regard embryoid body formation and TC as the gold standards indicating desired properties (as is often done and advocated so far) but as indicative of properties that the cells should not have. The catalogue of desired properties of the cells, which so far includes (regulated) proliferative properties and a defined differentiation potential, should be amended in the sense that their developmental (early embryonic pattern formation) potential ought to be of a restricted type (or, better, be totally missing). The challenge for research must be to define the most appropriate molecular targets within the canon of data recently provided by experimental embryology (Tam and Loebel, 2007; ten Berge *et al*., 2008). This asks for a re-thinking of strategies that have been established in many laboratories around the world so far, no doubt. But it is possible to envisage this change of focus now, and the advent of iPSC forces us to do so for ethical reasons. The chance is there and should be used to implicate this into legislative initiatives towards liberalization of stem cell research planned in a number of countries.

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