Cell Stem Cell
Meeting Report

Breakdown of the Potentiality Principle and Its Impact on Global Stem Cell Research

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Totipotency, defined as the ability of a single cell to generate an entire individual, has traditionally served as a cornerstone to frame the moral relevance of nascent human life. This "potentiality principle" has served as an ethical reference point for shaping legal regulations for stem cell research in most Western countries. Based on heterogeneous ethical, religious, and political views, different countries cope with recent advances in mammalian cloning and reprogramming in a remarkably diverse manner. This and related issues were key topics at a recent meeting held in Berlin, Germany, on ethical aspects of stem cell research in Europe. An emerging view from this event is that international heterogeneity in stem cell politics and legislation must be overcome in order to develop this field toward biomedical application.

The workshop "Ethical Aspects of Stem Cell Research in Europe," held April 19–20, 2007, in Berlin, was a joint effort of EuroStemCell and ESTOOLS, two large multinational research consortia funded by the 6th framework program of the European Commission (EC), and coordinated by Austin Smith (University of Cambridge, UK) and Peter Andrews (University of Sheffield, UK), respectively. In addition to projects focusing on stem cell biology, both consortia include work packages dedicated to ethics and societal issues related to stem cell research. These ethics projects are led by Goran Hermeren from Lund University, who was also one of the workshop organizers. The meeting brought together ethicists, philosophers, stem cell biologists, clinicians, biotech entrepreneurs, EC representatives, and politicians from all over Europe to confront controversial aspects of legislation on stem cell research, to evaluate the feasibility of stem cell-based therapies, and to define potential milestones for a road map toward clinical application. This report discusses the key scientific, ethical, and legal issues, with a particular focus on (1) the impact of the latest advances in cellular reprogramming on the concept of cell potency and (2) the regulatory challenges for translational stem cell research.

No Longer a Matter of Debate: The Need for Pluripotent Cells

In the past, discussions about stem cell research typically centered around the question of whether embryonic stem cells (ESCs) or somatic ("adult") stem cells represent the most suitable candidates for cell replacement therapies and other biomedical applications. Indeed, both types of stem cells feature unique properties and yielded an impressive series of recent advances. However, at the meeting it became clear once again that the initial enthusiasm about an alleged transdifferentiation of adult stem cells has given way to a more realistic perspective, i.e., using adult stem and progenitor cells for repairing their tissue of origin.

The therapeutic potential of adult stem cells was highlighted by impressive work on Duchenne muscular dystrophy that was presented by Giulio Cossu from the San Raffaele Scientific Institute in Milano. Pediatric patients afflicted with this still untreatable disease typically become wheelchair bound by their early teens and die in their early 20s due to respiratory insufficiency. Cossu and his colleagues succeeded in deriving and expanding a canine vessel-derived stem cell termed mesangioblast, which restored motility when injected intra-arterially into dystrophic dogs (Sampaolesi et al., 2006). Only a few months later, the Cossu team isolated the same stem cells from human donors, including pediatric Duchenne patients. The cells could be engineered to express human minidystrophin and were shown to colonize skeletal muscle of dystrophic immunodeficient mice (Dellavalle et al., 2007). This work provides perspectives to use either heterologous HLA-matched cells from healthy donors or autologous, gene-corrected cells from Duchenne patients for first clinical trials.

Already advanced into transition to a clinical application are Yann Barrandon and his colleagues from the Ecole Polytechnique Fédérale de Lausanne, who make use of ex vivo expanded autologous keratinocytes for transplantation in third-degree burn wounds. This life-saving treatment has been shown to restore essential barrier functions of the skin and result in a normal epidermis being functional for many years.

While these studies show how close adult stem cell research has come to clinical application, it is fair to note that recent excitement about these cells was not as much fueled by the idea of tissue-specific repair but rather

by the vision of using them as an ESC-like donor source for a large variety of cell types. Core of this concept was the idea to use adult stem cells from regenerative tissues such as bone marrow to generate cells of nonregenerative tissues such as nervous system, heart, or insulin-producing cells. With sound evidence supporting "transdifferentiation" into functional neurons, cardiomyocytes, and β cells still lacking, the adult stem cell field is experiencing a sobering yet healthy period of refocusing.

Notwithstanding recent evidence from rodent studies suggesting that stem cells in the adult brain can give rise to new neurons (Thored et al., 2006), Olle Lindvall (Lund University), a pioneer in the field of neural transplantation, pointed out that it is unclear to what extent the endogenous adult stem cell source can be tapped and exploited for clinical purposes. For the time being, Lindvall stresses, ESCs provide the most versatile exogenous donor source for neural replacement. Indeed, the potential of pluripotent ESCs for the in vitro generation of nonregenerative cell types is increasingly recognized.

The Rush for Alternatives: Loophole Science and True Breakthroughs

Ever since ESCs entered the public debate, the derivation of these unique cells from the early embryo spurred moral concerns and fueled an at times misguided quest for less ethically controversial alternatives. Transdifferentiation hype and multipotent adult progenitor cells with ESC-like plasticity provided the first wave. "From blood to brain" and the like were frequent slogans in these days. Envisioning the potential to generate diverse somatic cells from the patient to be treated, these reports also tackled the problem of immune rejection of allogeneic cells. Advocates for pluripotent stem cells responded with the vision of therapeutic cloning, building on Campbell and Wilmut's seminal work. However, as first exemplified by the sheep Dolly, somatic nuclear transfer (SNT) into enucleated oocytes yields totipotent cells, which, in terms of potentiality, represent an embryo equivalent. In addition, translation into a clinical realm would require large numbers of donated oocytes, which constitutes an ethical problem of its own, as underlined by Anne McLaren from the Wellcome Trust Gurdon Institute in Cambridge (see also McLaren [2007]).

The controversy spurred a remarkable and unprecedented series of studies aimed at circumventing these sensitive points. Cell fusion, which originally brought down the concept of transdifferentiation, was now turned into an avant-garde technology for transferring pluripotency from ESCs to somatic cells. And while the tetraploidy of the resulting cells remains an issue, this area of research is likely to provide a wealth of data on reprogramming mechanisms. Other scientists aim at disengaging totipotency. Conditional inactivation of cdx2, for example, prevents trophoblast differentiation of a cloned cell, thus excluding implantation and further development (Meissner and Jaenisch, 2006). "Do no harm" was the credo of others, culminating in studies on human blastomere-derived ESCs (Klimanskaya et al., 2006). Since preimplantation diagnosis has shown that single blastomeres can be removed without incapacitating the embryo, this strategy was proposed as an ethically acceptable route to derive human ESCs. Loophole science? Davor Solter, from the Max Planck Institute for Immunobiology, argued that at least some of these studies might be confronted with the question of whether they would have ever been conducted for purely scientific reasons. And in any case, none of these attempts seem to have really solved the deepest ethical concerns. For example, in the case of altered nuclear transfer based on cdx2 ablation, the scientifically and morally challenging question has been posed of whether a cdx2-deficient clone is an embryo that died when it needed cdx2, or a purely artificial entity that never started to live in the first place. This brings us back to biological potency, one of the central themes of the meeting.

Potentiality and Reprogramming

In a nutshell, the potentiality argument as applied in the context of hESC research mandates that, since a zygote has the potential to become a person, it is warranted to treat it as if it were already a person, and hence that it is wrong to use it for the derivation of hESCs no matter what potential benefits they may entail. It is important to scrutinize the validity of this claim because several legislations in Europe are based, more or less explicitly, on the potentiality argument. Germany is a paradigmatic example: the embryo protection law states that an embryo (the object of the law's protection) is a fertilized zygote, as is every totipotent cell separated from it. Hence, as further sustained by Ludger Honnefelder (Institute of Science and Ethics, University of Bonn), it becomes a criminal act to use totipotent cells for cell derivation or any other intervention, which is not to the benefit of the embryo.

Now, sustained bioethical scrutiny had done away with the potentiality principle a long time ago, demonstrating that it is a prime example of non sequitur: if A has the potential to become B, it does not follow that we can or in fact should treat it as if it were already B. We may decide to treat B as if it were A, and we may even show good reasons for doing it, but we cannot consider this decision as the only possible logical conclusion since it is simply a political act. As such, it cannot be just taken at face value and needs to be justified, on a par with all political acts. The importance of this conclusion is general and lies not so much in the fact that it debunks the potentiality argument per se as in the way in which it shows how regulations can no longer be justified as emanating directly from "facts," be they moral or biological facts. In other words, when we recognize that the fact that the zygote is totipotent does not logically or necessarily imply that we should treat it as a person, we also recognize something much more general, namely that laws and regulations are primarily social constructions, even or especially when, as in the case of natural law, they purport to act neutrally as repositories of naturally encoded notions of good and bad. But if this is true, why then are we still dealing with the potentiality principle, and why do many

countries still use it as a resource to articulate their policies around the life sciences? On this issue, the Berlin meeting provided some key insights. The point is that, whereas bioethical reasoning had long dismissed the validity of the potentiality argument, science still seemed to provide, until a few years ago, a set of reasonable boundaries to treat cell potency as if it were a natural given. But the birth and life of Dolly, along with the flurry of research on genome reprogramming, have since demonstrated how fragile and ultimately inadequate the concept of potency is to inspire public policy (Stanton and Harris, 2005). As Hans Schöler from the Max Planck Institute for Molecular Biomedicine and Giuseppe Testa from the European Institute of Oncology pointed out, potency without context means nothing. In many ways, this had always been true: the IVF zygote, totipotent in abstract terms, still needs a woman and her womb in order to deploy its potency. But the latest advances in genome reprogramming research, on the wave of last year's seminal paper by Yamanaka, make this point even more vivid. The work showed that overexpression of a defined combination of genes can turn fibroblasts into ES-like cells (Takahashi and Yamanaka, 2006). Three new papers now extend these findings, showing, among other data, that such induced pluripotent stem cells (iPS cells) are germline competent and, after injection into tetraploid blastocysts, can even generate purely iPS cell-derived embryos (Maherali et al., 2007; Okita et al., 2007; Wernig et al., 2007). The overall conclusion is that potency is best understood as a fluent state, which can be initiated in any cell through the steering of selected gene networks. In this sense, it is indeed an ironic twist of fate that the research efforts aimed at reprogramming adult cells in order to bypass the perceived ethical problems of ES cells end up dismantling the very argument most of those ethical problems were based on. For if we were serious about the potentiality principle, we would, in the not-too-distant future, also have to prohibit the use of artificial but totipotent derivatives of somatic cells generated by cellular reprogramming. But exactly this implausible conclusion identifies the moral implications of reprogramming research: if through the manipulation of selected genes a differentiated cell can be coaxed back into a totipotent state, this transient state will have lost any intrinsic property and hence any possible moral claim. It will just be *one* of many possible states in which a cell can exist, within or outside the human body.

From Proof-of-Principle to Translation: Who Is Doing What?

Basic scientists easily get excited about the potential benefits of their work for biomedicine. Yet it remains a long way from a beating cardiomyocyte generated in a cell culture dish to a standardized treatment of heart disease. One important question addressed at the meeting was how to plan first clinical trials. Key prerequisites for clinical applications are safety and efficacy. A central issue associated in particular with the use of ESC-derived somatic cells is to transplant them at high purity and devoid of remaining undifferentiated cells, which may otherwise lead to tumor formation. Moreover, standardized procedures based on good manufacturing practice (GMP) are required for scale-up and differentiation of stem cells in a clinical context. Most of the currently existing human ES cell lines were generated in a nonstandardized manner, in some cases using procedures known to promote acquisition of genetic aberrations. Outi Hovatta, from the Karolinska Institute, pointed out that the stem cell field needs to move toward the generation of additional ES cell lines under standardized conditions. The establishment of stem cell banks would enlarge the spectrum of available haplotypes and thus facilitate donor-host matching and reduce the risk of transplant rejection.

Once the scientific and basic technical requirements for donor cell generation are sorted out, there will remain a need to actually engage in product development and distribution. GLP and GMP facilities, automatic systems to scale-up stem cell production, stem cell banking, and safety testing do not fall within the realm of academic research. These tasks can only be tackled in joint efforts with the commercial sector. At this stage, translation can be further enhanced by close association of biotech industry, academic research labs, and university hospitals, as it is already the case in Sweden. Lars Wahlberg, from NsGene A/S, stressed that access to academic gene and cell therapy facilities may significantly ease the implementation of clinical trials. On the other hand, Greg Stewart, from Medtronic, Inc., pointed out that investing in SC production is extremely expensive, and the profit of a company is unlikely to be satisfactory when compared to the amount of money and effort required to build up the business. One question emanating from this controversy was whether temporary governmental support could accelerate this process and provide incentives for biotech industry to take on such high-risk endeavors. This issue becomes particularly relevant for treatment of orphan diseases, in which costs for research and development vastly exceed potential profits.

Ethics' Paralyzing Grip on Commercialization

Many at the meeting agreed that usually an ambivalent attitude results when industry faces clinical applications. Whereas biomedical stem cell research is considered a high-ranking goal, commercialization generally is met with skepticism. And while academic stem cell research enjoys increasing support throughout Europe (with the notable exceptions of Germany and Italy), the potential for commercial use has become the true battlefield of stem cell critics. If parts of society see it as morally problematic to use embryos for research, how much more problematic is this process when substantial commercial revenue comes into play? Wherever such fundamental questions of moral and human value are raised, the ensuing polarization tends to become a minefield for politicians and decision makers. Commonly, the best way to survive in a minefield is not to move. But as several contributors to the Berlin meeting underscored, not moving also has a cost: the stigmatizing attitude toward commercial stem cell

research has stalled much progress that will be required for developing biomedical applications. And if one is serious about the ethical appraisal of stem cell research, one should at the very least also consider the ethical costs associated with delaying the fruition of stem cell research's potential benefits.

The situation is particularly difficult when voters ask for both technological leadership and conservation of traditional views. The Bush administration has managed to navigate this conflict with the de facto creation of two parallel universes, a straightforward task given the standing of free enterprise in American tradition: while federally funded hESC research is tightly regulated and restricted to cell lines generated before August 2001, the private sector is practically not subject to any restrictions.

One of the most controversial topics in the field of translational stem cell research is patenting, as pointed out by Aurora Plomer from the University of Sheffield. In Europe, research on human ES cells is accepted, yet in most cases patents are not. For instance, in Germany, academic research on human ES cells is permitted, and public funding bodies explicitly demand researchers to assess patentability of their results prior to publication. Yet, a recent lawsuit challenging a patent for the derivation of neural precursor cells from ES cells that was originally granted to Oliver Brüstle in 1999 is a tough reminder of the current contradiction in this area of regulation. As Clara Sattler (Max Planck Institute for Intellectual Property, Competition, and Tax Law, Munich) explained, the German federal patent court ruled that, despite the ethically high-ranking goals pursued, the patent is immoral and against the "ordre public" due to the embryonic origin of the cells. In line with the previous considerations, several commentators at the Berlin meeting questioned the very notion of ordre public as well as the peculiar arrangement whereby patent courts are entrusted with the authority to determine, on behalf of society, what ultimately constitutes ordre public. On a practical level, then, countries like Germany will continue to lose ground to countries that forbid patents on the actual derivation of ES cells, but not on essential aspects of their subsequent manipulation (as is the case of the Brüstle patent). Thus, Goran Hermeren stressed, the risk of patenting abroad discoveries made in such European countries, as was already the case for several technological advances, persists.

International Regulations Needed to Facilitate Synergy

One of the most remarkable observations a visitor of the Berlin meeting could be confronted with was the pronounced international heterogeneity in stem cell legislation. Projects that are perfectly legal in Sweden and the UK can draw a 3 year prison sentence in Germany. This incongruency creates a plethora of problems for international collaboration. Despite common funding by the 6th and 7th frameworks of the EC, scientists within Europe cannot freely exchange personnel and cell lines. Researchers from countries with very restrictive legislation, such as Germany, might even become liable by taking on coordinating positions within European networks comprising institutions that generate their own hESC lines. The meeting made evident that these problems severely hamper international collaboration and synergy development, which are instrumental in a multidisciplinary field such as stem cell research. There is an increasing awareness that European heterogeneity in stem cell politics is about to slow the development of stem cell-based biomedical applications and further impede the competitiveness of Europe in global stem cell science. Yet, in the Berlin meeting, these issues were not only discussed from the point of view of global competition. There was also intense discussion about the ethical implications of individual countries' stance that de facto withholds the development of novel biomedical applications from their populations. The question of whether such restrictive legislations will be able to live up to this ring fencing once new stem cell-based treatments become available remains for the most unsolved.

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