EMBO Molecular Medicine

The promise and the challenge of modelling human disease in a dish

Human embryonic stem (hES) cells are already transforming our vision of regenerative medicine and cell replacement therapies because of their unique ability to maintain themselves indefinitely and to form all cell types in the body. hES cell lines with genomes that are predisposed to disease can be used to develop cellular models of human pathologies. The generation of disease-specific and/or genotypically diverse human stem cell lines that can differentiate into many cell types will have great value for understanding the biology of the cell types affected in disease and analysing disease mechanisms, screening for drug candidates that can slow or prevent disease-related degeneration as well as toxicological testing and understanding the variation between patients in their responses to therapeutics. In addition, to prepare for cell-based therapies, new technologies for the generation of stem cell lines that are histocompatible with or specific to individual patients will provide a strategy to overcome the challenges of immune rejection.

» The iPS technology is the source of much optimism . . . «

Several different methods have been developed to make hES cells; to date, these all require the use of human female egg cells as a starting point. Recently, several laboratories have reported that adult cells from human skin and other sources can be induced to revert back to earlier stages of development and exhibit stem cell-like properties. The methods for 'reprogramming' have been developing rapidly but generally involve putting multiple genes into skin cells and then exposing them to specific chemical environments tailored to hES cell growth. While these cells appear to have a developmental potential that is similar to that of hES cells, they are not derived from human embryos. To distinguish these reprogrammed cells from the embryo-sourced hES cells, they are termed induced pluripotent stem (iPS) cells. The iPS technology is the source of much optimism; iPS cells appear to have the same applications in terms of regenerative medicine as hES cells with the added advantage that they can potentially be generated from any particular individual, whatever its age or condition, without the need to use or create fertilized eggs. Hence, iPS research is not subjected to the same limiting ethical controversies and restrictions as hES work. Basic iPS technology is still evolving, however, and, for many diseases, patient-specific iPS cells have not yet been developed. All of these encouraging, yet early-stage approaches fall under the new field of regenerative medicine.

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Much of the recent spectacular progress made in this field was based directly on work using mouse lines. We should encourage the continued development of mouse stem cell-based resources and projects since they retain several key advantages. As compared to their human counterparts, they grow and differentiate faster, can be genetically modified more easily, and can be generated from lines of mice expressing tissue-specific fluorescent markers. Moreover, there is no problem in generating control cell lines with an identical genetic background. These cells are especially useful and versatile in terms of high-throughput screening (HTS) applications, where they can be used more effectively to identify therapeutic targets and drug candidates. Subsequent validation can then be performed on patient-specific human lines. Therefore, these mouse and human cell lines represent an important complementary tool for advancing human stem cell biology. Developmental biology and the last 20 years of cell and molecular biological advances in development are also at the basis of these emerging technologies.

» HTS on iPS cell-based models of disease is already feasible. «

Of critical importance for the utility of stem cells is their reliable and efficient differentiation into specific cell types such as disease-linked types of neurons or blood lineage cells. Techniques for directed differentiation of stem cells into specific neuronal or blood cells are invariably based on reconstructing in the culture dish the sequential conditions that lead during normal development to the generation of the cell type in question. Current technology allows us to reprogram genetically healthy or disease-linked skin cells into iPS cells and then to coax them to become motor neurons or cardiac myocytes. As discussed, iPS cells can be used in a variety of studies involving testing corrective gene therapy, in transplantation studies in animal models of disease or in HTS paradigms to find small molecules that modify the disease-related phenotype. The ability of reprogrammed cell types to rescue disease phenotypes will serve as a proof of principle for future therapeutic treatment of human disease.

While human iPS cell-based treatments are still very far from becoming a reality, HTS on iPS cell-based models of

disease is already feasible. HTS has been at the heart of pharmaceutical drug discovery for many years. Classically, it has relied on the prior identification of a single therapeutic target, *e.g.* an enzyme or a kinase whose inhibition was known to have a favourable effect on disease outcome. Purified target molecules were isolated individually and collections of millions of different chemical compounds were tested one by one for their ability to inhibit the target. In spite of some key successes, however, there has been a gradual realization that many disease processes do not represent isolated targets and that the behaviour of even a given target is different in a cellular context. There has therefore been a growing recognition of the importance of screening for drug candidates that can correct defects in the cell types affected by the disease. However, the bottleneck is that many particular cell types are not available.

» Stem cell research in neuroscience has potential at multiple levels. «

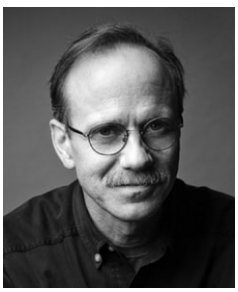
This is especially true for neurological diseases; clearly, human neurons cannot be isolated from living human patients and even their mouse equivalents are available in very limited quantities. Stem cell derivatives provide a remarkably well-adapted solution to this problem. Stem cells can be derived from human patients or mouse models, expanded *ad infinitum*, and then differentiated into the cell type affected in the disease under study. If these disease-related cells can be shown to have a cellular or molecular phenotype that is related to the disease, they can be used to develop a screening assay and subsequently, collections of thousands of different compounds can be screened to identify those that prevent the disease phenotype. By identifying the proteins targeted by the compounds, new details about the disease mechanism can be inferred. Moreover, molecules with drug-like properties can be selected for further optimization and *in vivo* testing, so that their potential as lead candidates for future drug development can be evaluated.

Examples of applications of these strategies can be found in Neuroscience. Stem cell research in neuroscience has potential at multiple levels. It can inform us in unique ways about the developmental mechanisms that are involved in constructing the specific neural circuits that allow us to think, feel and move. Moreover, stem cell technology provides a new and exciting means with which to study the mechanisms of disease, including currently incurable diseases such as Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis (ALS; also known as Lou Gehrig's disease). One example of an area of neuroscience research that is using the basic biology of cell-type specific differentiation to better understand human disease is the study of motor neuron development. Motor neurons are the spinal cord cells that cause muscles involved in breathing, swallowing and walking to contract. Therefore, in diseases such as ALS, the progressive loss of motor neurons leads to paralysis and death of the patient. Until recently, it was not possible to gain access to the cells affected in the spinal cord of human patients. Stem cell biology has radically changed this situation. We can now use ES and iPS cells to create the distinct classes of motor neurons that innervate different muscles in the body and we can ask how they differ and why some are resistant to ALS. Thanks to iPS cell-derived human motor neurons, for the first time we have direct access to motor neurons with the same genetic make-up as the ALS patients from which they were derived. This means that we can model the ALS disease process in the culture dish, allowing us to better understand the disease mechanisms and screen for new drugs that can prevent motor neuron degeneration.

As outlined above, there are many reasons for optimism for this new approach to modelling human disease, but there is clearly a long way to go before we can really claim success. Along with the optimism there is considerable skepticism about this new strategy.

» EMBO Molecular Medicine will play an essential role in disseminating and scrutinizing the progress being made in this exciting area of regenerative medicine. «

Some of the key issues of concern are: (1) can authentic differentiated cell types that reflect the cells of the disease be generated *in vitro*? (2) Many diseases manifest their phenotype later in adulthood, so how can one hope to generate a meaningful phenotype in a reasonable time in culture? (3) Reprogrammed cells from somatic cells carry with them many of the genetic scars that accumulated through life and each individual and each cell is different, so will not the variance overwhelm the ability to detect the phenotype or mechanism that represents authentic features of the disease? Although there are very few examples of clear success so far, what is apparent is that many young, and not so young, scientists are focusing their interests and efforts in this direction. In addition, federal, state and local governmental funding agencies are pouring funds into this area based on promise and hope. We can only anticipate that the exponential growth this field is currently experiencing will continue for many more years to come and we are certain that EMBO Molecular Medicine will play an essential role in disseminating and scrutinizing the progress being made in this exciting area of regenerative medicine.



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