Lecture

2013 Runme Shaw Memorial Lecture: Clinical Applications of Stem Cells in Modern Medicine—21st Century and Beyond

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The rapid advancement of biomedical research in the recent years was propelled by a series of groundbreaking technological inventions and breakthrough discoveries. In this lecture, I will discuss about the scientific achievements that led to different eras of intense research with profound impact on biomedicine, focusing on the use of stem cells for research and clinical applications.

Molecular Biology Revolution

One of the most striking breakthroughs in biomedicine is the discovery of the deoxyribonucleic acid (DNA) structure by Watson and Crick. The understanding of the structure and inheritance of the genetic blueprint pioneered the molecular biology revolution. Genes were cloned, sequenced, studied and modified. It led to an unprecedented acceleration of our understanding of the gene and its products at an incredible resolution not previously known to mankind. In the molecular biology era, gene products for therapeutic uses were also manufactured. For example, the sequencing of the insulin polypeptide and the cloning of its cDNA enabled the production of large quantities of pure recombinant insulin for the treatment of diabetes. These scientific activities generated tremendous impact to the society in terms of knowledge, health and economy.

Genomics Revolution

The genetics of an individual influence different aspects of the human physiology, from physical traits to mental traits to disease susceptibility. One of the technologies that were developed in the molecular biology era is the Sanger sequencing method. Using this method, the monumental project of sequencing the human genome was completed over the course of more than 10 years. The draft of the human genome was published in 2000, and it sparked the birth of the Genomics revolution. Analysis of the correlation between genetic variants and human phenotypes has been a major research theme. Using high throughput DNA analysis approaches, genome wide association studies have led to numerous new insights into human physiology and disease.

Development of improved sequencing technologies now brings genomics research to an even higher level. Capillary-based sequencing technology was replaced by sequencing by synthesis technology, which allows for even higher throughput. More recently, single molecule-based sequencing was also developed. Today, with the current commercially available sequencing instruments, it is possible to sequence a human genome within 2 to 3 days. Concurrent with the revolution in sequencing technology, the cost of sequencing has also reduced dramatically over the past 10 years. All these developments have led to the commodification of sequencing; while in the past, only the major sequencing centers could afford a suite of sequencing instruments, the continuous drop in sequencing costs enables more deployment and application of genomics in a clinical setting. The generic way to treat patients under the assumption that everyone has equivalent response to a drug can be changed with the implementation of new genomics technologies. Advances in the Genomics era ultimately contribute to achieving the vision of Genomics Medicine / Personalised Medicine, which entails the customisation of healthcare with medical decisions, practices, and products being tailored to the genetic profile of individual patients.

Stem Cell Revolution

The global phenomenon of ageing has led to an increase in diseases associated with the elderly, many of which are due to a degeneration of physiological functions. One emerging strategy is to develop methods to replace the lost function by introducing cells or their cellular products to enhance or mediate regeneration. Arising as a promising source of these cells and cellular products for regenerative medicine are stem cells. Stem cells are undifferentiated or 'blank' cells found in the human body that are capable of perpetuating themselves as stem cells, and have the potential to develop into many different cell types that carry out various functions. Different types of stem cells have been isolated and they can be generally categorised into embryonic stem cells and tissue-specific stem cells. Tissue-specific stem cells can be isolated from the adult

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body and they can be found in a variety of different tissues such as skin, brain and liver. I would like to highlight 2 applications of tissue-specific stem cells.

One striking example is the use of stem cells to re-engineer a whole organ for transplantation to replace damaged tissues. In 2008, it was reported that a 30-year-old patient who suffered from tuberculosis had a malfunctioned airway and needed a windpipe replacement. The doctors took a donor windpipe, decellularised it and reintroduced stem cells from the patient to reconstruct the living parts of the windpipe. The windpipe graft was then transplanted back to the patient. The operation was reported to be a great success as the reconstructed windpipe was no different from the patient's own airway tissues and no signs of immune rejection were detected. The patient was able to lead a normal life up to this day. Even after years, this first tissue engineered windpipe remained functional, well vascularised and had normal ciliary function and mucus clearance. The lung function and cough reflex were also normal.[1]

A second example of stem cell application is the use of cord blood stem cells for treating human diseases. In 2006, cord blood was successfully used to treat a young patient with Severe Combined Immunodeficiency which rendered the boy unable to overcome infections. After a successful cord blood transplant at the KK Women's and Children's Hospital, the patient is now in good health with a functional immune system.[2]

Despite these successful applications of stem cells in the clinic, most tissue-specific stem cells cannot be robustly expanded in the laboratory. Active research is ongoing to identify ways to expand tissue-specific stem cells.

On the other hand, stem cells can also be derived from early embryos. Professor James Thomson first reported the successful derivation of human embryonic stem cells from donated human blastocysts.[3] These cells have several important properties. First, they can be passaged stably in the laboratory for extended periods of time. Hence, they can serve as a renewable source of cells. Second, they are pluripotent and retain the ability to differentiate into cells of the 3 major lineages (endoderm, mesoderm and ectoderm). By changing culture conditions, human ES cells can be differentiated into neurons, hepatocytes, cardiomyocytes or other cell-types. These human cells can therefore be used for biomedical research and regenerative medicine. Singapore's first step in the human embryonic stem cell arena was pioneered by the work from Professor Ariff Bongso and his team who first isolated stem cells from human preimplantation blastocysts in 1994.[4]

The biology and applications of pluripotent stem cells are spearheaded by research performed using mouse embryonic stem cells which were derived much earlier than human embryonic stem cells. Mouse embryonic stem cells can be genetically engineered and used to create genetically modified mice, allowing for the generation of previously unavailable animal models for human diseases. Mice are now widely used as the experimental models for researchers to study gene functions in vivo. These groundbreaking works have led to the awarding of the Nobel Prize in Physiology or Medicine 2007 to Professors Mario R Capecchi, Sir Martin J Evans and Oliver Smithies for their discoveries of principles for introducing specific gene modifications in mice with the use of embryonic stem cells.

From the developmental biology point of view, pluripotent stem cells are also used to study early development of animals and humans. To impact clinical research, it would be necessary to derive patient-specific human embryonic stem cells. However, the derivation is technically difficult and it is also challenging due to ethical issues as human blastocysts are required.

The Birth of the "Reprogramming" Era

For a very long time, it was believed that it is not possible to convert mammalian fibroblasts, a terminally differentiated cell-type, into stem cells with a higher differentiation capacity. However, in 2006, it was demonstrated that the introduction of a simple cocktail of 4 transcription factors into mouse fibroblasts can facilitate the formation of colonies of cells that resemble mouse embryonic stem cells within 3 weeks.[5] These cells were termed "induced pluripotent stem cells". In 2007, there were at least 3 independent reports on the conversion of human fibroblasts into human induced pluripotent stem (iPS) cells. The technology was so straightforward that it was quickly reproduced in different laboratories worldwide. For the very first time in biology, patient-specific pluripotent stem cells can be generated easily in laboratories. This work, in conjunction with the work on demonstrating somatic cell nuclear transfer, had earned Sir John B Gurdon and Professor Shinya Yamanaka the Nobel Prize in Physiology or Medicine 2012 for the discovery that mature cells can be reprogrammed to become pluripotent.

A new field of "iPS cells" was then created. Since 2006, there are over 500 papers published on the topic. Singapore's first paper on iPS cells was published in 2009.[6] This paper described a new combination of factors for reprogramming. In 2010, researchers from Singapore identified a new transcription factor that can improve the quality of iPS cells.[7] The prospect of generating patient-specific iPS cells opened up many new research opportunities. It is now possible to use a patient's cells for disease modelling to investigate disease mechanisms and to screen for new or existing drugs.

Engineering the Human Genome for Gene Correction or Gene Therapy

A technology that enhances the use of stem cells for research is DNA editing. By using nucleases that recognise defined DNA sequences to mediate DNA cleavage, scientists can now effectively edit and engineer the genomic sequence of a cell. This technology encompasses the use of zinc finger, TALE and Cas9 proteins. To illustrate the potential of this technology in future medicine, I will highlight 2 examples. In the first example, zinc finger nucleases were used to direct the correction of the F9 gene to achieve a phenotypic correction of haemophila B in a mouse model.[8] In the second example, zinc finger nucleases were used to disrupt the CCR5 gene which encodes for the HIV co-receptor. The CD4+ T cells with mutated CCR5 gene became resistant to HIV-1 infection.[9]

What are the Major Challenges for Pluripotent Stem Cell Research?

Coupled to DNA sequencing and genome correction, the iPS cell technology allows access to individual genetics. Despite the tremendous progress in the generation of iPS cells, there are important considerations for the clinical use of these cells. First and foremost, iPS cells are not the magic bullets to all diseases. More research in the coming years would still be needed to improve our ability to differentiate pluripotent stem cells into cells that resemble the real functional cells in the body. There is also a new research frontier to generate minimal organ units from stem cells. For example, there is already good progress in transforming human embryonic stem cells into organ units such as the optic cup in the laboratory. The human embryonic stem cell-derived neural retina is able to grow into a multi-layered tissue that contains both rods and cones.[10]

Applications of Pluripotent Stem Cells

There are 2 major applications of pluripotent stem cells in biomedicine. First, the different cell-types that can be obtained from pluripotent stem cells can be used in drug development and human disease modelling. Cardiomyocytes derived from patient's iPS cells have been used for toxicity, safety and disease modelling studies. Cellular differences that were detected between the neurons derived from normal and Parkinson's disease patients have also enabled a better understanding of disease progression. These studies were only feasible with the development and utilisation of iPS cell technology.

The second major application of pluripotent stem cells is to use them as a source of differentiated cells for cell-based therapy. The area which is most developed is the use of retinal pigment epithelium cells for treating patients with advanced dry age related macular degeneration or Stargardt's macular dystrophy. Safety trails are currently conducted by Advanced Cell Technology[11] to test the sub-retinal transplantation of retinal pigment epithelium cells derived from human embryonic stem cells. This year, the Japanese government also approved the world's first clinical trial on the use of human iPS cell-derived retinal pigment epithelium on patients with age related macular degeneration. Even though the iPS cell technology is relatively new, the stem cell field is rapidly pursuing research to advance the different applications of pluripotent stem cells in biomedicine.

Technological Advances Open up Unprecedented Opportunities for Modern Medicine

In summary, for the past 10 years, we have witnessed the development of very powerful technologies for studying the human systems which was not feasible before. The 3 major technologies are: (i) low-cost DNA sequencing, (ii) iPS cell technology, and (iii) genome editing. Today's technologies on 23 August 2013 enable the prospect of personalised genome sequencing, personalised iPS cell generation and personalised genome correction. The research done at the Genome Institute of Singapore, A*STAR, is directed towards this vision. A human genome can be sequenced within 48 hours, a human iPS cell-line can be generated within a month, and a human genome can be corrected in the laboratory within a month. While these technologies are still under development, the future prospect is exciting.

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REFERENCES

1. Gonfiotti A, Jaus MO, Barale D, Baiguera S, Comin C, Lavorini F, et al. The first tissue-engineered airway transplantation: 5-year follow-up results. Lancet. pii: S0140-6736(13)62033-4. doi: 10.1016/S0140-6736(13)62033-4.
2. Lee. Mind Your Body: Saved by Stem Cells. The Straits Times, 2 December 2010.
3. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, et al. Embryonic stem cell lines derived from human blastocysts. Science 1998;282:1145-7.
4. Bongso A, Fong CY, Ng SC, Ratnam S. Isolation and culture of inner cell mass cells from human blastocysts. Hum Reprod 1994;9:2110-7.
5. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126:663-76.
6. Feng B, Jiang J, Kraus P, Ng JH, Heng JC, Chan YS, et al. Reprogramming of fibroblasts into induced pluripotent stem cells with orphan nuclear receptor Esrrb. Nat Cell Biol 2009;11:197-203.
7. Han J, Yuan P, Yang H, Zhang J, Soh BS, Li P, et al. Tbx3 improves the germ-line competency of induced pluripotent stem cells. Nature 2010;463:1096-100.
8. Li H, Haurigot V, Doyon Y, Li T, Wong SY, Bhagwat AS, et al. In vivo genome editing restores haemostasis in a mouse model of haemophilia. Nature 2011;475:217-21.
9. Perez EE, Wang J, Miller JC, Jouvenot Y, Kim KA, Liu O, et al. Establishment of HIV-1 resistance in CD4+ T cells by genome editing using zinc-finger nucleases. Nat Biotechnol 2008;26:808-16.
10. Nakano T, Ando S, Takata N, Kawada M, Muguruma K, Sekiguchi K, et al. Self-formation of optic cups and storable stratified neural retina from human ESCs. Cell Stem Cell 2012;10:771-85.
11. Advanced Cell Technology. Available at: http://www.advancedcell.com/ Accessed 18 December 2013.