Ena Ray Banerjee

Perspectives in Translational Research in Life Sciences and Biomedicine

Translational Outcomes Research in Life Sciences and Translational Medicine, Volume 2

Springer

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ISBN 978-981-10-5869-1 ISBN 978-981-10-5870-7 (eBook)
DOI 10.1007/978-981-10-5870-7

Library of Congress Control Number: 2017947844

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Perspectives in Translational Research in Life Sciences and Biomedicine

Preface

When a student of Life Sciences (or Biosciences or Biological Sciences) subject embarks on a career path, the first choice may be education and the second research. Education is passing on acquired knowledge and Research is generating new knowledge. An important aspect of Biological Sciences especially the classical subjects like Zoology, Botany and Physiology, along with the newer disciplines of Microbiology, Molecular Biology, Biotechnology and Immunology, is to understand life as we know it. Taxonomy helps classify the life forms and Biology aims to study them. Health Sciences is a follow-through of this same study pertaining to human life. Medical Sciences and Anthropology are the other disciplines that study intervention strategies in health and disease and human biology, prehistory, cultural aspects and evolutionary aspects through present day, respectively. I have always felt, as a student of Zoology, that the pattern of the structural and functional aspects of life events can be studied. I was introduced to the concept of Systems Biology much later. That all forms of life have some advice to offer, as is celebrated in the Vedas and Upanishads of the ancient Hindus, is amply exemplified as I travelled the long road from classical taxonomy, the basics of the functional units of life cells and Cell Biology, the technical aspects of engineering the cell and its parts in Molecular Biology and ultimately my specialized training in Immunolobiology and Stem Cell Biology and then the translational aspects namely Inflammation Biology and Regenerative Medicine, respectively. The aspects that need to be mentioned are technology, prior knowledge and present context of the research, proposal points, that is where we wish to start, why (rationale of the study), expected outcome and where we go from the lessons learnt—these make up the patchwork quilt of understanding basic principles of life and living forms so that they may become viable processes or products, bench-to-bedside health sciences aspects, missing nuances of fundamental threads of life that can lead to technology platforms and ecological aspects that can be analogous both from the perspective of within the living form and outside it. Connected and discrete systems thus take form from apparently disorganized and disparate melee of living organisms that share this planet with us. Questions ought to be simple and ways to find answers simple as well. Technology ought to be applied as per the need to derive answers, troubleshoot, find alternate strategies if the original one fails and so on, and so forth. This book ought to give some answers in tissue engineering and validation in preclinical models;

drug discovery efforts using eco-compatible probiotics, nanoparticles, polymers and peptides; and understanding signatures of behaviour of living forms at the molecular and cellular level for appropriate intervention. Green technology exploration, using advanced assays for quantitative and qualitative assessment and determination of therapeutic, prophylactic, maintenance, diagnostic or cosmetic uses of food in addition to nutrition, remains a key mission of Translational Outcomes Research Group. Biodiversity exploration not only validates usefulness of the same but also ensures their conservation. Biosurvey, bioprospecting and bioresource generation remains a mandate of the Life Sciences practitioners so that appreciation of living forms and translation of that powerful knowledge may strike a sustainability on this planet.

Kolkata, India Ena Ray Banerjee

About the Author

Dr. Ena Ray Banerjee is Professor of Zoology in University of Calcutta, India and heads the Immunobiology and Regenerative Research Unit of the department. Her interests are manifold but centred around translational outcomes research in life sciences by exploring biodiversity through bioprospecting and converting them into bioresources currency. Alumnus of the premier educational institutions Lady Brabourne College and Gokhale Memorial Girls' school, Dr. Ray Banerjee has trained in Immuniobiology during her Ph.D. and worked extensively in Immune modulation in inflammation in general and cytokine mediated inflammation in particular in Indian Institute of Chemical Biology, under Center for Scientific and Industrial Research. She has taught under- and post-graduate Zoology with a special emphasis on Endocrinology and Immunology under University of Calcutta, India. She then pursued her post-doctoral studies as visiting scientist and subsequently faculty of University of Washington School of Medicine, Seattle, USA in Pathobiology, Hematology, and Allergy and Infectious diseases departments. Her work on target identification and validation in asthma and other related lung diseases helped her transition from a completely academic pursuit to a more applied one. She began with immunological studies defining key molecules in inflammation and eventually super-specialized into lung inflammation particularly allergy and made a natural transition onto regenerative medicine of the lung, having worked with some renowned names in the field. Her work validated several key targets in hematopoiesis and inflammation and also pioneered tissue engineering of lung lineage-specific cells of the non-ciliated variety from Human embryonic stem cells and identified stem cell niches in mouse lung. She returned to India and worked in a leadership role in a drug discovery company where her team led drug discovery efforts in inflammation, especially pertaining to the lung using rigorous structure activity correlation studies to reject or recommend pharmacological molecules working in tandem with Chemistry and Pharmacology departments. Her experience of working in India, in the US, in academia and industry prepared her for a unique role—that of translational research in life sciences. With this aim she returned to the renowned University of Calcutta to leverage her unique training in academia and industry,

fundamental and applied research, she began her activities developing technology intensive processes or products with a core knowledge of Zoology. Whether developing animal models of diseases for screening novel drug entities or models to understand fundamental life processes such as developmental biology, or looking for bioresources from local biodiversity, her group works on drug discovery efforts using novel drugs (small molecules), herbal extracts (functional food), probiotics (neutraceuticals), novel antibody-mediated (camelid antibody) and cells (tissue engineering of stem cells of embryonic origin, adult tissue origin and umbilical cord-derived) in inflammatory disease models (tissue-specific inflammation in the lung and systemic inflammation) and degenerative disease models. She has published widely in premier scientific journals and her publications are widely cited in 'methods' volumes as well as 'drug discovery' websites and portals. She is also respected as an academician and educationist *par excellence* and has spearheaded the rejuvenation of a world class heritage museum because she believes that to do bioprospecting and molecular drug discovery, knowing and respecting your biodiversity is key. Through her efforts, this archived faunal repository is positioned to become a centre of excellence for technology-based capacity building and an important educational interpretive centre.

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Tissue Engineering and Cell-Based Therapy in Regenerative Medicine

1

Tissue Engineering is an offspring of the field of biomaterials development, a more sophisticated method of using biomaterials, which are materials used in medical devices. Tissue engineering involves the use cells, scaffolds and active molecules to build functional tissues. Tissue engineering aims to create working constructs that have the ability to rehabilitate and preserve damaged tissues or organs. Artificial skin and cartilage are examples of tissues that have been engineered.

The field of Regenerative Medicine includes the field of tissue engineering, along with the principle of self-healing. Here, the body makes use of its own systems, often with the aid of other biological supplements, to regenerate cells, tissues and organs. The terms 'tissue engineering' and 'regenerative medicine' have now become almost synonymous, as they rely on each other to completely remedy, instead of simply treat, complex chronic diseases.

Tissue engineering addresses research on the healing of wounds and cellular prostheses for the human body. It also includes the use of regenerative medicine to create tissues for drug discovery, using premature cells called stem cells or pluripotent cells.

There are four main elements necessary for the production of functional tissues using tissue engineering. These components include cells (stem cells from an embryo, foetus or adult, are often used), a matrix (a base for the cells to grow on; it can be a permanent or temporary scaffold), supplementary biomaterials (like cytokines or other signalling molecules) and a bioreactor (an apparatus with an environment suitable for the growth of the cells).

1.1 Current Status of International Research on Tissue Engineering of Stem Cells into Lungs

1. In 2012, researchers from Boston University, Darrell Kotton, Tyler Longmire, and Laertis Ikonomou, in collaboration with various scientists, like Hans-Willem Snoeck of New York's Mount Sinai Hospital and Jay Rajagopal of Massachusetts General Hospital, took almost a decade to successfully grow a lung using stem cells. For years, scientists have been inducing embryonic stem cells to develop into mature tissues. Tissues like muscle and nerves have been quite easily grown. On the other hand, tissues that originate from the endoderm, like liver, lung, thyroid and pancreas are more difficult to grow. When the embryo is 3 weeks old, it forms the endoderm, and in 5 weeks, it differentiates into organs. During this time, the endoderm differentiates into organs like the lungs and stomach. The steps that are involved in the differentiation of the endoderm into the lung have till now eluded researchers. Scientists created a knock-in reporter gene which glowed green when the differentiating stem cells expressed a gene called Nkx2-1. This helped them track the development of the lung tissue. To ensure that Kotton and his team had actually grown lung cells, they

E.R. Banerjee, *Perspectives in Translational Research in Life Sciences and Biomedicine*, DOI 10.1007/978-981-10-5870-7_1

collected samples of mouse lungs and washed them with detergent to make them cell-free systems. They then used one lung with 15-day old home-grown lung cells, and the other lung with undifferentiated stem cells as control. Within 10 days of seeding, the 15-day old lung cells had colonized the lung and established themselves in a pattern that is recognized as lung tissue. On the other hand, the cells in the control lung did not develop into lung tissue, but grew into an indistinguishable cluster.

2. 2013 saw the successful development of human stem cells into working cells of the lung and airway by researchers from the Columbia University of Medical Center (CUMC). They successfully obtained Type II alveolar epithelial cells, expressing surfactant protein B, by the differentiation of human embryonic stem cells (hESCs). Building on Dr. Snoeck's study in 2011 that there are a plethora of chemical factors that induce the differentiation of hESCs or induced pluripotent stem cells (iPSCs) into the endoderm, they found new factors that induce the differentiation of hESCs or iPSCs into working lung epithelial cells. The developed cells expressed markers of both lung and airway epithelial cells, especially Type II alveolar epithelial cells. These cells are involved in maintaining the lung alveoli, and also in the repair of the lung after injury. These findings have led to the possibility of developing autologous lung grafts, to treat diseases like idiopathic pulmonary fibrosis. Developing this type of graft would involve taking a damaged lung, removing the cells from it, leaving behind only the lung framework or scaffold, and then seeding it with new lung cells developed from the patient himself. This would remove the possibility of rejection.

3. Dr. Cheryl Nickerson and her team of researchers from the Arizona State University Biodesign Institute, made use of a technique called Dynamic Suspension Culture to repopulate cadaveric lung scaffold with cells. In this technique, they placed the cells and a lung scaffold, obtained from deceased individuals, in a bioreactor that constantly blends them together, increasing the incorporation of the cells into the scaffold. This study shows that this technique can be used for ex vivo lung engineering. The advantage of a dynamic culture is that it improves cell growth, viability and stimulates the cells to differentiate, compared to static cultures. This method of using cadaveric lungs in dynamic suspension culture to develop functional lung tissues may eventually resolve the obstacle caused by a dearth of organs for transplant. This technique can help patients who are affected with Chronic Obstructive Pulmonary Disorder (COPD), a global disease affecting over 60 million people.

4. A group of researchers from the Perelman School of Medicine at the University of Pennsylvania and from the Duke University, have discovered that lung tissues have a certain flexibility that allow them to repair and regenerate the damage caused after injury. The aim of this study was to find out how mature lung cells are activated to repair damage in response to any injury, so that the process can be triggered in conditions like COPD.

The alveoli are made of two types of airway cells-Type I cells, where gases are exchanged, and Type II cells which produce surfactants to help keep the airways open. These cells, though have distinct functions, can transform into each other. The team found that these two types of cells had a common ancestral precursor stem cell, present in the embryo. Though it had already been shown that Type I cells could be developed from Type II cells by differentiation, the opposite had not been reported yet. They used a mouse model to demonstrate the interchangeability of the cells. They found that Type I cells differentiated into Type II cells in around 3 weeks. This study showed that even specialized cells, considered to have lost the ability to differentiate, could revert to its earlier state under suitable conditions. The team hoped to use this technique to treat other lung conditions like acute respiratory distress syndrome (ARDS) and idiopathic pulmonary fibrosis (IPF).

5. Dr. Frank McKeon, Dr. Wa Xian and their team of researchers from the Jackson Laboratory have studied the role played by some stem cells in the regeneration of injured lungs. They have found, in a mouse model system, a type of adult

lung stem cell called p63+/Krt5+. These cells are present on the distal airways. When cultured, these cells formed alveoli-like structures. On infection with H1N1, these cells infiltrated to the sites of inflammation, and assembled into sac-like structures. These structures are similar to alveoli, both visually and molecularly. They found that mice lacking these stem cells in their lungs could not counter the H1N1 infection, and their lungs showed scarring and defective oxygen-exchange functions. This showed that these p63+/Krt5+ cells play an important role in the regeneration of lung tissues.

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1.2 Current Status of National Research on Tissue Engineering of Stem Cells into Lungs

1. Constructing a functional scaffold from cadaver tissues, and using them in regeneration of tissues, has been an obstacle for scientists working on tissue engineering. A team, led by Sweta Gupta, tried to make a scaffold from goat lung, and to test its biocompatibility for use in tissue engineering. They decellularized sections of goat lung, and seeded HEPG2 cell lines into it. They found the seeded cells to retain over 99% of their viability. The scaffold matrix was also found to be biocompatible, providing a tool for tissue engineering applications.
2. In 2011, the Drugs Controller General of India (DCGI) gave permission to Stempeutics Research, a company based in Bangalore, to carry out Phase-II clinical trials to find the efficiency of stem cell therapy, using the company's flagship product Stempeucel. They were to administer the stem cell therapy to patients for 6 months, analyse the results and monitor the patients for 2 years. The therapy was to be tested on patients with liver cirrhosis, diabetes, COPD and osteoarthritis. Stempeutics was hopeful of the results, and aimed to release the first stem cell based drug by 2013.
3. Stem Cure Pvt. Ltd., a private company at Ahmedabad, is carrying out research on lung diseases, focusing on Interstitial Lung Disease (ILD) and pulmonary hypertension (PH). It has been found that stem cells can be used to treat the degeneration occurring in ILD. Recent studies have found that administered cells have a paracrine effect, and regulate local inflammatory and immune responses.

Stem Cure Pvt. Ltd. assists institutes, clinics and hospitals in stem cell therapy of ILD and PH.

Reference

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1.3 Different Techniques of Tissue Engineering for Induction of Differentiation of Pluripotent Stem Cells

2006 saw researchers from Kyoto University, Japan, determine some environments that could induce and genetically alter adult cells (induced pluripotent stem cells or iPSCs) to behave like stem cells. Certain genes necessary for maintaining the main characteristics of ESCs were introduced into the iPSCs to induce them into an ESC-like state. Despite the need of further research, researchers now have started to concentrate on the potential use of iPSCs in drug discovery and transplantation medicine. This focus has been triggered by the idea that a patient's own cells could provide him with an unlimited supply of immune cells. Cells lose their pluripotency as they progress through development, as they become more restricted to their ability to differentiate. Most cells completely differentiate into mature cells. However, stem cells with limited potency stay in certain parts of the body, like bone marrow, skin and intestine, and serve as a source for cell replacement. The differentiated cells are stable, and neither do they generally transform into other types of cells, nor do they revert to their own undifferentiated forms. This review summarizes the progress made in the field of iPSCs, emphasizing on the mechanisms of cellular reprogramming and its application in cell therapy.

Findings about induced pluripotency brought about the union of scientific technologies and principles that were developed over the last 60 years. The challenges involved in isolation, culture, purification and differentiation of tissue-specific stem cell lines have urged researchers to look for alternative techniques to 'create' pluripotent cells from existing non-pluripotent cells. Nuclear Reprogramming is one such technique, where a stable change is induced in the nucleus of a mature cell, and this change is maintained and replicated in the daughter cells formed after mitosis. Other such techniques include somatic cell nuclear transfer (SCNT), altered nuclear transfer (ANT) and fusion of somatic cells with ESCs.

The nuclear reprogramming technique helps in the creation of iPSCs, which involves introducing, into the mature somatic cells, genes that encode transcription factors. These transcription factors regulate other genes involved in embryonic development. In a study in 2006, it was found that only four transcription factors—Sox2, Oct4, Klf4 and c-Myc, could reprogram mouse fibroblasts to an ESC-like state. These factors were known to maintain pluripotency. In 2007, researchers used these four genes, along with Nanog and Lin28, in several combinations, to derive iPSCs from human cells. Eventually, researchers managed to generate iPSCs from somatic tissues of rat and monkey.

Several strategies have been explored to enhance the efficacy, and to reduce the harmful side effects, of reprogramming. Further studies have narrowed the number of genes needed for reprogramming, thereby making the process more specific. Chemicals that can substitute or improve the efficiency of the transcription process are being identified. These discoveries are making the technique of reprogramming more specific and simple, thus paving the way for the development of patient-specific stem cells for clinical application.

Some factors have to be taken into account before reprogramming cells. The process of reprogramming has a number of obstacles in the

field of regenerative medicine. Before considering reprogramming as a clinical tool, the efficacy of the process has to be enhanced. Despite the identification of the numerous molecular pathways involved in the reprogramming of somatic cells, further research is still needed to pinpoint the full range of occurrences that guide the process. 2006 saw Takahashi and Yamanaka succeed in reprogramming somatic cells into pluripotent cells, by transforming adult mouse fibroblasts into iPSCs. They did this by the ectopic expression of certain transcription factors. This technique was subsequently optimized by several research groups, also demonstrating that iPSCs were actually similar to ESCs. Direct reprogramming was done in human cells in 2007, by Takahashi et al. and Yu et al., which was a breakthrough in the field of regenerative medicine. Although the generation of iPSC lines is theoretically easy, in reality it is a slow and ineffectual process comprising a large number of unknown events. To ensure reproducibility in the production of iPSCs, several variables must be taken into account. These variables include the choice of soluble factors used for reprogramming, the mode of delivery of these factors, the type of cell, parameters, like timing and level of expression of these factors, the conditions under which the iPSCs are derived, and the methods to identify and characterize the reprogrammed cells. This has been summarized in Fig. 1.1, and each step has been dealt with in detail.

1. Choice of reprogramming factors: There are four main transcription factors which mediate reprogramming. These are Oct4, Sox2, c-Myc and Klf4, as identified by Takahashi and Yamanaka in 2006. These factors have been found to work in various cell types of mouse, rhesus monkey and humans. These four genes have been used in various combinations for the reprogramming of the cells. It has been found that in mouse fibroblasts, the activity of Sox2 can be replaced by Sox1 and Sox3, but with lower efficacy. It has also been seen that Klf4 can be replaced by Klf2, and c-Myc by L-Myc and N-Myc. Even though the original set of factors is considered a standard for reprogramming, other small molecules and

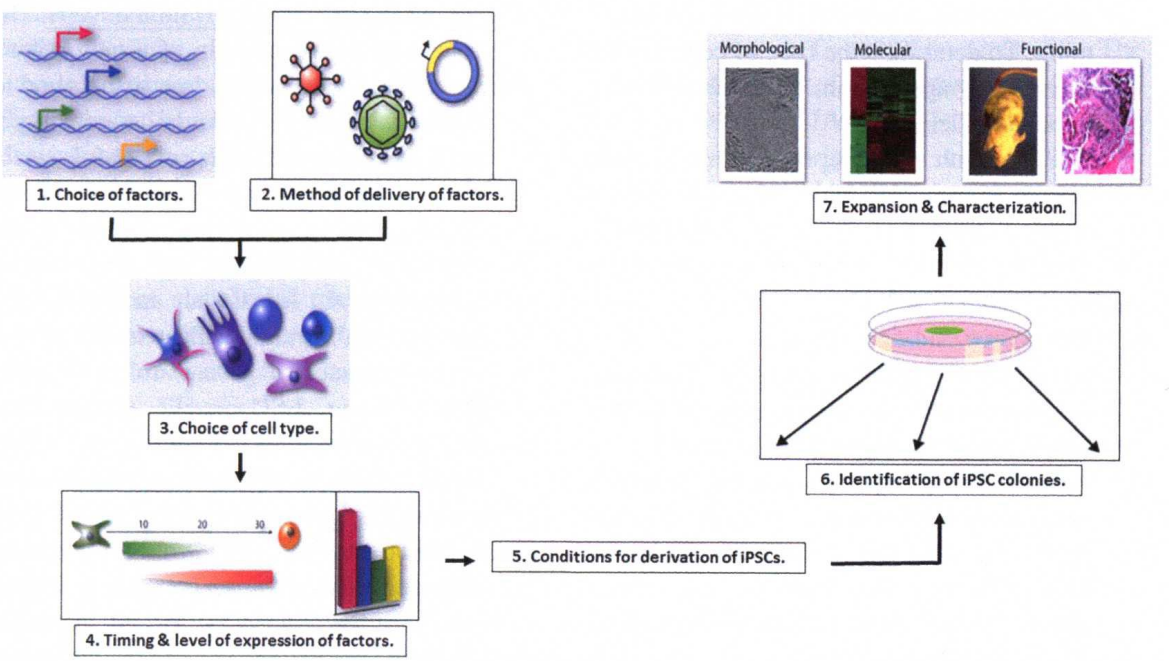

Fig. 1.1 Overview of the process of derivation of iPSC (Ref: Guidelines and Techniques for the Generation of Induced Pluripotent Stem Cells, by Nimet Maherali & Konrad Hochedlinger)