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This dissertation, "Establishment and Direct Differentiation of Induced Pluripotent Stem Cells From a Hirschsprung's Patient" by Sum-yee, Jasmine, Yung, □□□, was obtained from The University of Hong Kong (Pokfulam, Hong Kong) and is being sold pursuant to Creative Commons: Attribution 3.0 Hong Kong License. The content of this dissertation has not been altered in any way. We have altered the formatting in order to facilitate the ease of printing and reading of the dissertation. All rights not granted by the above license are retained by the author. Abstract: Hirschsprung's (HSCR) disease is a congenital disorder in which some enteric ganglion cells are absent in the colon due to incomplete colonization of neural crest cells (NCCs) in the hindgut, causing chronic constipation. A significant number of HSCR patients also clinically present with other NC- associated disorders, such as ventricular and atrial septal defects (VSD/ASD). A hypomorphic allele or SNP of a major gene, RET, causes or imparts susceptibility to HSCR. In particular, SNP (rs2435357) residing in the intron 1 of RET gene was found to be highly associated with HSCR and lead to reduced RET expression. However, the molecular basis of syndromic HSCR with VSD/ASD is largely unclear. In our project, with the use of the induced pluripotent stem cell (iPSC) technology, we aim to establish a patient-specific model unravel the etiology of HSCR and the associated disorders. To this end, 3 iPSC clones from a syndromic HSCR patient with VSD/ASD, carrying the RET risk allele in rs2435357 were generated. We attempted to use different protocols to directly differentiate iPSCs into NCCs with unique HOX expression patterns, corresponding to anterior cranial/vagal or posterior vagal/trunk NCCs. Consistently, the patient iPSCs displayed similar capacities in generating NCCs at all axial levels, marked by HNK-1 and □p75□ DEGREESNTR. Nevertheless, the patient NCCs and their derivatives exhibited severe migration and/ or differentiation defects in making neurons and smooth muscle cells. In particular, HNK-1+□p75□ DEGREESNTR+ HOX+ (vagal/trunk) NCCs derived from patient-iPSCs were less migratory compared to the control NCCs, while no obvious migration defect was observed in their cranial counterpart, indicating that the migration defect was only restricted to the more posterior NCCs. In addition, these patient NCCs were less capable in generating neurons and readily biased toward generating glial cells. Intriguingly, the neural differentiation defects were restricted to NC lineage. The capacity of patient iPSCs to make various types of CNS progenitors and neurons was comparable to that of the control iPSCs, nicely recapitulating the patient's phenotype where only enteric neurons, but not CNS progenitors were affected. Subsequent expression analysis revealed that patient NCCs express lower level of RET which is known to be regulating enteric NCC migration and differentiation. Whole transcriptome RNA sequencing analysis also revealed an enhanced expression of genes associated with gliogenesis and a reduced expression in genes associated with neurogenesis and migration. Moreover, the expression of a new candidate gene ALDH3B1 was shown to be significantly reduced in the HSCR-iPSC-derived NCCs that might contribute to the disease pathogenesis. In summary, these data suggests that reduced RET expression in HSCR patient NCCs may at least partly account for the disease phenotypes. DOI: 10.5353/th_b5435660 Subjects: Stem cells Hirschsprung's disease - Etiology Stem cells have the ability to differentiate into all types of cells within the body, thus have great therapeutic potential for regenerative medicine to treat complicated disorders, like Parkinson’s disease and spinal cord injury. There will also be many applications in drug development. However, several roadblocks, such as safety issues and low efficiency of pluripotent stem cell (PSC) line derivation need to be resolved before their clinical application. This thesis focuses on these two areas, so as to find methods to overcome the limitation. It covers deriving embryonic stem cells (ESCs) from several different species and reports an efficient system to generate induced pluripotent stem cells (iPSCs), and the first iPSC mice in the world. The results in this thesis confirm that somatic cells can be fully reprogrammed with the four Yamanaka factors. In addition, we have found that the Dlk1-Dio3 region can be a potential molecular marker to distinguish the fully reprogrammed iPSCs from partially reprogrammed ones. All of these results will help improve the safety of PSCs in the clinical applications and increase the current low induction efficiency of their production. Maintaining Embryonic Stem Cells and Induced Pluripotent Stem Cells. Induced Pluripotent Stem Cells: Therapeutic Applications in Monogenic and Metabolic Diseases, and Regulatory and Bioethical Considerations. This book represents an updated overview on selected topics related to mesenchymal stem cells as well as induced pluripotent stem cells. The book is divided into three main sections that cover several topics including: sources of both stem cell types, their preparation and general properties, as well as their therapeutic indications and clinical utilization with particular attention given to their use in infectious diseases, osteoarthritis, as well as immunological disorders. This volume captures the rapid developments in the field of induced pluripotent stem (iPS) cells, which have provided novel opportunities and approaches both for better understanding a number of human diseases and for developing new platforms for drug development and screening for such diseases. Specifically, representative protocols on various disease models have been collected from labs around the world. Written for the highly successful Methods in Molecular Biology series, chapters include introductions to their respective topics, lists of the necessary materials and reagents, step-by-step, readily reproducible laboratory protocols, and tips on troubleshooting and avoiding known pitfalls. Authoritative and practical, Patient-Specific Induced Pluripotent Stem Cell Models: Generation and

Characterization is an ideal reference for scientists working on furthering iPS research. Brain diseases can have a large impact on patients and society, and treatment is often not available. A new approach in which somatic cells are reprogrammed into induced pluripotent cells (iPS cells) is a significant breakthrough for regenerative medicine. This promises patient-specific tissue for replacement therapies, as well as disease-specific cells for developmental modeling and drug treatment screening. However, this method faces issues of low reprogramming efficiency, and poorly defined criteria for determining the conversion of one cell type to another. Cells contain epigenetic “memories” of what they were that can affect reprogramming. This book discusses the various methods to reprogram cells, the control and determination of cell identity, the epigenetic models that have emerged and the application of iPS cell therapy for brain diseases, in particular Parkinson’s disease and Vanishing White Matter (VWM). This detailed volume presents a series of protocols that are representative of recent developments and improvements in induced pluripotent stem cells (iPS cells) and corresponding human disease models. Reflecting the latest technology for generating induced pluripotent stem cells (iPS cells) and their initial characterization, the book explores techniques invaluable both for studies of disease-specific cell types and for their potential applications in regenerative medicine. Written for the highly successful Methods in Molecular Biology series, chapters include introduction to their respective topics, lists of the necessary materials and reagents, step-by-step and readily reproducible laboratory protocols, as well as tips on troubleshooting and avoiding known pitfalls. Authoritative and practical, Induced Pluripotent Stem Cells and Human Disease: Methods and Protocols serves as a vital guide that is valuable for not only experts but also novices in the stem cell field. Generation of Human Induced Pluripotent Stem (iPS) Cells from Liver Progenitor Cells by Two Chemicals and the Clinical Application. This lavishly-illustrated, authoritative atlas explores the intricate art of culturing human pluripotent stem cells. Twelve chapters – containing more than 280 color illustrations – cover a variety of topics in pluripotent stem cell culturing including mouse and human fibroblasts, human embryonic stem cells and induced pluripotent stem cells, characteristic staining patterns, and abnormal cultures, among others. Atlas of Human Pluripotent Stem Cells in Culture is a comprehensive collection of illustrated techniques complemented by informative and educational captions examining what good quality cells look like and how they behave in various environments. Examples of perfect cultures are compared side-by-side to less-than-perfect and unacceptable examples of human embryonic and induced pluripotent stem cell colonies. This detailed and thorough atlas is an invaluable resource for researchers, teachers, and students who are interested in or working with stem cell culturing. Human Testis-Derived Pluripotent Cells and Induced Pluripotent Stem Cells. This extensive new edition presents protocols reflecting the great strides made in the study of induced pluripotent stem (iPS) cells. The collection explores new and improved methods for the generation, expansion, and maintenance of iPS cells from different tissue types, characterization of their differentiation pathways along different lineages, and their potential utility in tissue repair and regeneration. Written for the highly successful Methods in Molecular Biology series, chapters include introductions to their respective topics, lists of the necessary materials and reagents, step-by-step, readily reproducible laboratory protocols, and tips on troubleshooting and avoiding known pitfalls. Comprehensive and up-to-date, Induced Pluripotent Stem (iPS) Cells: Methods and Protocols, Second Edition aims to arm stem cell biologists, both novice and expert, with invaluable protocols that are currently being used in various laboratories around the world. The series Advances in Stem Cell Biology is a timely and expansive collection of comprehensive information and new discoveries in the field of stem cell biology. Recent Advances in iPSC-derived Cell Types, Volume 4 addresses how different cell types can be derived from induced pluripotent stem cells. Somatic cells can be reprogrammed into Induced pluripotent stem cells by the expression of specific transcription factors. These cells are transforming biomedical research in the last 15 years. The volume teaches readers about current advances in the field. This book describes the use of induced pluripotent stem cells to form different cell types which can be used in cell therapy as well as to model several diseases in vitro, enabling us to study the cellular and molecular mechanisms involved in different pathologies. In recent years, remarkable progress has been made in the obtention of induced pluripotent stem cells and their differentiation into several cellular populations, tissues and organs using state-of-art techniques. This volume will cover what we know so far about the use of iPSCs to derive different cell types, such as: erythroid cells, mucosal-associated invariant T cells, megakaryocytes, cerebral cortical neurons, inner ear cell types, airway epithelial cells, male germ cells, trophoblasts, cardiomyocytes, ßpancreatic cells, and more. The volume is written for researchers and scientists interested in stem cell therapy, cell biology, regenerative medicine, and organ transplantation; and is contributed by world-renowned authors in the field. Provides overview of the fast-moving field of induced pluripotent stem cell technology, regenerative medicine and therapeutics. Covers the following cell types derived from iPSCs: erythroid cells, mucosal-associated invariant T cells, megakaryocytes, cerebral cortical neurons, inner ear cell types, airway epithelial cells, male germ cells, trophoblasts, cardiomyocytes, ?pancreatic cells, and more. Contributions from stem cell leaders around the world Induced Pluripotent Stem Cells as a Source of Hepatocytes. The series Advances in Stem Cell Biology is a timely and expansive collection of comprehensive information and new discoveries in the field of stem cell biology. iPSCs - Novel Concepts, Volume 15 addresses how important induced pluripotent stems cells are and how can they can help treat certain diseases. Somatic cells can be reprogrammed into induced pluripotent stem cells by the expression of specific transcription factors. These cells have been transforming biomedical research over the last 15 years. This volume will address the advances in research of how induced pluripotent stem cells are being used for treatment of different disorders, such as liver disease, type-1 diabetes, Parkinson’s disease, macular degeneration of the retina and much more. The volume is written for researchers and scientists in stem cell therapy, cell biology, regenerative medicine and organ transplantation; and is contributed by world-renowned authors in the field. Provides overview of the fast-moving field of stem cell biology and function, regenerative medicine and therapeutics Covers spinal cord injuries, type-1 diabetes, liver disease, Parkinson’s disease, graft vs. host disease, and much more Contributed by world-renown experts in the field Because of the huge potential of human embryonic stem (hES) cells, especially the newly developed human induced pluripotent stem (hiPS) cells, in disease treatment and life quality improvement, enormous efforts have been made to develop new methodologies to translate lab discoveries in stem cell research into bed-side clinical technologies. In Human Embryonic and Induced Pluripotent Stem Cells: Lineage-Specific Differentiation Protocols, experts in the field present a comprehensive collection of protocols designed for labs around the world. The topics covered in this detailed volume include techniques used for maintenance of hES and iPS cells in either small or large scale, techniques for directing hES and iPS cell lineage specification, techniques for enhancing the maturity of differentiated hES and iPS cells within three-dimensional scaffolds, techniques for reprogramming adult cells into iPS cells, techniques for generating patient-specific iPS cells, and techniques for translating hES and iPS cell research into new therapies. Chapters include lab ready protocols with tips on troubleshooting and avoiding known pitfalls. Wide-ranging and authoritative, Human Embryonic and Induced Pluripotent Stem Cells: Lineage-Specific Differentiation Protocols will be a tremendous aid for researchers and students who wish to explore these areas and transform their discoveries into the next generation of regenerative medicine and tissue engineering technologies. Induced Pluripotent Stem (iPS) cells are mature cells that have been genetically reprogrammed so that they return to their embryonic state. It is not yet known if iPS stem cells and embryonic stem cells differ significantly. Today many fundamental belief systems in biology are shifting towards accepting that mature body cells can be reverted to an embryonic state without the help of eggs or embryos. With their changed identities, iPS cells are then ready to serve as new tools for research in the fields of disease pathogenesis, drug discovery, oncology, and cell transplantation. One example of this would be using iPS from a patient’s mature cells to repair damaged tissue; it is thought that there would be very low incidence of rejection of the ‘new’ tissue in these cases. For the last four years, this therapeutic promise has been studied by hundreds of researchers worldwide in an effort to understand the ability of these cells to reverse their biological clocks.

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