Preface

Human pluripotent stem cells such as human embryonic stem cells (hESC) and induced pluripotent stem cells (iPSC) with their unique developmental plasticity hold immense potential as cellular models for drug discovery and in regenerative medicine as source for cell replacement. While hESC are derived from a developing embryo, iPSC are generated with forced expression of key transcription factors to convert adult somatic cells to ESC-like cells, a process termed reprogramming. This is a rather appealing choice for generating pluripotent stem cells since it overcomes ethical issues concerning the use of developing embryos and iPSC can be generated from patient-specific or disease-specific cells for downstream applications. The purpose of the book "Pluripotent stem cells: Methods and Protocols" is to highlight the best methods and systems for the entire work flow.

A key concern with iPSC is that randomly integrated transcription factors used during reprogramming can lead to genomic instability and altered epigenome. In order to generate safer iPSC, several advances have been made in the development of footprint-free systems for the delivery of transcription factors. However, some of the currently available footprint-free reprogramming methods are more efficient or consistent than others in producing iPSC from diverse somatic sources. The advantage in having the capability to generate iPSC from diverse somatic cells is that it offers a choice to utilize convenient sources of patient material such as peripheral blood or altered somatic cell sources in case of disease patients where peripheral blood may not be an ideal choice. Part I focuses on this aspect with reviews and articles on ESC derivation and iPSC generation.

An additional key consideration for the culture of safer, therapy-compliant cells is the choice of media and matrix used for the generation and expansion of cells. Traditionally, pluripotent stem cells are cultured on murine embryonic fibroblasts in media systems that contain serum or serum replacement. The biggest challenge in routine and large-scale culturing of these cells is the effort and technical expertise required to maintain the pluripotent stem cells in their undifferentiated and karyotypically normal state. In order to distill the art of culturing these cells to a predictable science, several media systems that are serum free, feeder free, and chemically defined have been developed and successfully used. This, in combination with matrices that eliminate the use of feeder cells, paves the path towards more defined and regulated culture systems. Further, use of different reprogramming methods from diverse somatic cell types cultured under varying conditions can often lead to iPSC clones that differ in their differentiation potentials. Identification of methods and streamlined protocols for directed differentiation into highly specialized cells is critical for efficient conversion of the pluripotent stem cells into the desired functional cell type. Part II summarizes media systems for expanding ESC and iPSC, with detailed protocols for directed differentiation into specific lineages.

A key unanswered question that is currently the focus of several studies is whether iPSC are truly pluripotent and equivalent to ESC. Traditional characterization have relied on cellular methods of pluripotent stem cell characterization such as observing morphology, differential

dye staining or specific antibody labeling. Furthermore, the teratoma assay is currently considered as the in vivo gold standard for pluripotency confirmation. However, the combination of transcriptome and epigenome analysis using new technology platforms is rapidly evolving and will enable high-throughput characterization and standardization of pluripotent stem cells. Some of the commonly used cellular and molecular characterization methods are compiled under Part III.

Finally, pluripotent stem cells are amenable to genetic labeling and can thus be used for dissecting basic biology, and for creating disease models and screening systems. Successful gene delivery, however, depends on easy cloning systems to rapidly generate vectors, gene delivery methods to deliver these vectors into pluripotent stem cells, and engineering platforms that meet the need for sustained context-specific gene expression. Part IV reviews the potential application of labeled stem cells with specific methods for cloning, gene delivery, and cell engineering.

Integrated work flow, familiarization to available methods and detailed protocols are critical for simplifying the complex technical challenges associated with pluripotent stem cell. Our intended goal and the objective of this book will be met when both novice and expert users can use this book as a comprehensive guide for the successful generation, culture, characterization, and differentiation of pluripotent stem cells.

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