Preface

The 1998 report describing the derivation of human embryonic stem cells (hESCs) set in motion an unprecedented wave of research and public discourse on the basic biology and the potential therapeutic application of these cells as well as the ethics surrounding their derivation and use. It seemed that everyone was interested in these cells derived from human blastocyst-stage embryos. The public debate and scientific interest continues today, 13 years later. Although there is no denying that the derivation of hESCs was a major breakthrough in our efforts to understand early human development and to treat human diseases, the science behind it was built on decades of solid research that began with the study of human and mouse teratomas and teratocarcinomas (see the reference list for a necessarily abridged compendium of seminal papers in this stem cell field).

Teratocarcinomas are the malignant form of teratomas, tumors that comprise a complex, disorganized mixture of cells and tissues representing cellular derivatives of all three of the embryonic germ layers. Importantly, the study of teratocarcinomas significantly increased our knowledge of embryonic development through pioneering work that led to recognition of the relationship between the differentiated cells and tissues that developed from the tumor and normal embryogenesis. Eventually, methodologies that allowed for the long-term in vitro culture of the stem cells of the teratocarcinomas were devised and, thus, the so-called embryonic (or embryonal) carcinoma cell (ECC) lines were established. Many ECC and other germ cell tumor lines were shown to be "pluripotent," which by definition means that they, like the tumors from which they were derived, were able to give rise to cellular derivatives of all three germ layers. Most of this groundwork in terms of basic culture protocols, basic characterization protocols, and basic in vitro and in vivo differentiation protocols developed using ECCs in the 1960s and 1970s was then used in early work to derive and characterize both mouse and human ESCs. In fact, the prototypical list of cell surface markers, TRA-1-60, TRA-1-81, SSEA-3, and SSEA-4, which are now commonly used to define hESCs as pluripotent, are the very same set originally used to define pluripotent human ECCs. The isolation of ECC lines from teratocarcinomas in the 1970s, therefore, provided the platform for the study of human pluripotent stem cells (PSCs), of which the hESC is but only one type.

While work was progressing on understanding the cause and nature of human teratocarcinomas, Stevens and Little at the Jackson Laboratory were developing a mouse model (the 129 strain) in which one could study the development and progression of teratomas in the mouse testis because this model had an unusually high occurrence of testicular teratomas. The 129 mouse strain ultimately became the strain of choice for those doing the methods development work that led to the derivation of ESCs and, in 1981, two reports describing the derivation of "embryo-derived pluripotent stem cells," which came to be known as mouse ESCs (mESCs), were published. Most of the mESC research during the 1980s focused on characterizing these diploid cells derived from the inner cell mass of the blastocyst. These highly unique cells were capable of prolonged in vitro culture, induced

differentiation down all three germ lineages in vitro and in vivo, and, most importantly, when injected into mouse blastocysts, contributing to the germline of chimeric mice. The subsequent development of technologies to target specific genes in mESCs gave rise to an entirely new way to study the function of mammalian genes through the generation of "knockout" mice.

The use of normal pluripotent stem cells (i.e., mESCs) that could be precisely manipulated in vitro and then placed back into a mouse embryo to give rise to a live animal carrying the precise mutation, which could be passed on to its offspring, changed the face of biomedical research – it provided a superior model in which to study the function of genes in the context of mammalian physiology. These technologies had such a major impact on biomedical research that the 2007 Noble Prize in Medicine and Physiology was awarded to Martin Evans, Mario Capecchi, and Oliver Smithies for their work developing the "principles for introducing specific gene modifications in mice by the use of embryonic stem cells."

In our view, three key technological advances led to the widespread use of mESCs for the study of mammalian gene function: (1) the discovery that leukemia inhibitory factor inhibited spontaneous differentiation, improving the ease with which these cells could be cultured; (2) the use of isogenic DNA targeting constructs, improving gene targeting efficiency and lowering the time and cost associated with producing mouse knockouts; and (3) the derivation, systematic banking, and distribution of new mESC lines, improving the availability, access, and quality of the cells available to researchers.

When we review the evolution of hESC research, we can draw some comparisons to the progression of mESC research, where the first decade for both was spent optimizing culture conditions and methods for derivation, characterization, and maintenance. As we move into the second decade of hESC research, we are almost daily being presented with new technologies that may move the field toward its promise as stated in Thomson's 1998 paper "that these cell lines should be useful in studying human developmental biology, drug discovery, and transplantation medicine." We suggest that three enabling technologies are at hand for human ESCs: (1) directed reprograming of somatic cells, which eliminates many of the ethical issues associated with the derivation and use of hESCs, increases genetic diversity of the available human PSC lines, and gives rise to better in vitro human disease models; (2) the discovery that a Rho-associated protein kinase (ROCK) inhibitor allows for efficient single-cell passaging and cryopreservation, increasing the efficiency and reliability of hPSC culture; and (3) defined, animal component-free media, which lay the groundwork for simplified scale-up for therapeutic applications, differentiation protocols, and toxicology screens (All of these technologies are well-described in this book).

When we pause to consider combining technologies, such as the production of induced pluripotent stem cells with next generation sequencing technologies, we can glimpse the future that may yield highly effective, personalized, medical treatments. Whether or not any or all of our hopes for the future of this research are fully realized, we know we are part of a dynamic scientific community that has at its core the desire to further human knowledge and improve the human condition.

We are, therefore, pleased to provide *Human Pluripotent Stem Cells: Methods and Protocols* to our scientific community. This book is a compilation of 33 detailed protocols in six categories of PSC research that cover laboratory essentials and the derivation of new PSC lines, including induced PSC lines, as well as their growth, maintenance, characterization, genetic manipulation, and differentiation. This book, of course, would not have

been possible without the generous contributions of our authors, all from leading research laboratories, who, working with their postdocs and students, have offered to share their validated and detailed protocols that we hope you, our colleagues, will find helpful in your own stem cell research programs.

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