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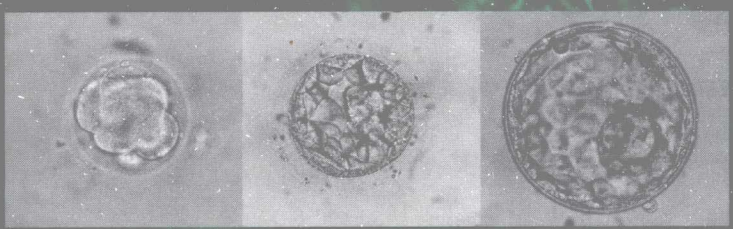
Human Embryonic Stem Cell Protocols

Edited by

Kursad Turksen



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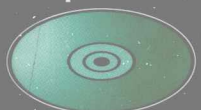

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Human Embryonic Stem Cell Protocols

Edited by

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Ottawa, Ontario, Canada

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Preface

Scientific research using human embryonic stem (hES) cells is one of the most controversial topics to come out of academic circles for some time. In fact, political and ethical controversies surrounding the study of hES cells have diverted many scientists from the field, thereby slowing its progress. Nevertheless, interest in understanding the regulation of their self-renewal capacity, commitment and differentiation along various lineages, as well as their potential utility in regenerative medicine applications, remains high. To facilitate the latter, there is a great need for the isolation of additional hES lines as well as the development of improved culture conditions to counter the view and practice that hES cells are difficult to maintain and use. I would therefore like to take this opportunity to thank all the contributors of this volume who have so generously shared their expertise and hard-won protocols.

I am grateful to Dr. John Walker for his support and encouragement during the process of compiling this protocol book. In addition, I would like to thank several others at the Humana Press for their support: initially Elyse O'Grady and Craig Adams and more recently Damien DeFrances. Also, I am grateful to Jennifer Hackworth for her wonderful support during the production of this volume.

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The tireless and always cheerful Tammy Troy has again been fantastic in helping to put together this volume.

Kursad Turksen

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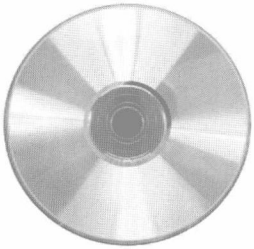

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Isolation, Maintenance, and Differentiation

Kursad Turksen and Tammy-Claire Troy

Summary

The isolation of pluripotent human embryonic stem (hES) cells having the capacity to differentiate in vitro to numerous cell types generated much excitement and promise in the field of regenerative medicine. However, along with great enthusiasm came hot controversy for stem cell research and researchers alike because available hES cell lines were isolated from "excess" embryos from in vitro fertilization clinics. Despite ethical and political debates, the methods and protocols to study diverse lineages are developing. Furthermore, strategies using specific growth factor combinations, cell–cell and cell–extracellular matrix induction systems are being explored for directed differentiation along a desired lineage. However, there is a great need to characterize the mechanisms that control self-renewal and differentiation and a necessity to improve methodologies and develop new purification protocols for the potential future clinical application of hES cells. After the scientific and political obstacles are overcome, it is anticipated that the hES cell field will make a tremendous difference in conditions, such as burn traumas and diabetic foot ulcers, as well a number of degenerative diseases such as Parkinson's disease, type 1 diabetes, rheumatoid arthritis, and myocardial infarction. In this introductory chapter, we will summarize and review recent progress in the field of hES cell differentiation protocols and discuss some of the current issues surrounding hES cell research.

Key Words: Embryonic stem (ES) cells; human embryonic stem (hES) cells; embryoid bodies (EBs); pluripotent; differentiation; in vitro differentiation; cell therapy; derivation; self-renewal; pluripotency.

1. Introduction

Embryonic stem (ES) cells possess two very important characteristics that have recently placed them at center stage for regenerative medicine: (1) the

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ability to proliferate without differentiation by a process of self-renewal and (2) the potential to form specialized cell types when induced to differentiation *(1)*. It has been a long-term objective of stem cell biologists to have a culture system whereby the ability to generate differentiated progeny from a continuously growing stem cell population in vitro would provide an arena for the study of stem cell/very early progenitor potential *(2,3)*. It would also make possible a comprehensive analysis of the underlying molecular mechanisms for the onset of cell lineage commitment and differentiation. With the increasing availability and utility of ES cells, some ground has been gained in this respect.

More than 20 yr ago, the first ES cells were derived from the inner cell mass (ICM) of 3.5-d-old mouse blastocysts *(4,5)*. When placed on a suitable fibroblast feeder layer in the presence of leukemia inhibitory factor (LIF), ES cells proliferate and remain pluripotent indefinitely *(4,5)*. It was also demonstrated that immortal stem cells could be manipulated in vitro, providing the opportunity to study early development as well as lineage potential of derived progenitors in vivo *(6,7)*. Since these pioneering studies, mouse ES cells have proved to be an excellent model system in which to study lineage commitment and progression in vitro *(8)*. Removal of ES cells from their feeder layer induces aggregation and differentiation into simple or cystic embryoid bodies (EBs). Simple EBs consist of ES cells surrounded by a layer of endodermal cells, whereas cystic EBs develop an additional layer of columnar ectoderm-like cells around a fluid-filled cavity, morphologically similar to embryos at the 6- to 8-d egg cylinder stage. The expression of markers for mesoderm (brachyury, activin), endoderm (collagen type IV), and ectoderm (α-fetoprotein) indicate that cells derived from all three germ layers occur in cystic EBs. At this stage, differentiation along several lineage pathways is possible with the appropriate inducing agents. Depending on culturing conditions, mouse ES cells have been shown to differentiate along a myriad of pathways including epidermal cells, type II alveolar epithelial cells, telencephalic precursors, osteoblasts, and cardiomyocytes, to name only a few *(9–14)*.

The very first established human ES (hES) cell culture was successfully isolated in 1998 from "unused" zygotes from an in vitro fertilization (IVF) clinic *(15)*. These and subsequent studies demonstrated that, as with mouse ES cells, hES cells have the capacity to self-renew without differentiation and are pluripotent in nature *(16–18)*. These characteristics impart great promise for tremendous impact on the future of regenerative medicine and medical research in general. In this overview chapter, we will provide a brief prelude to recent progress in the field of hES cell research before delving into the detailed protocol chapters within this volume.

2. Frontiers in hES Cell Derivation

IVF clinics assisting the reproduction of infertile couples have provided a source for the generation of several hES cell lines. In such clinics, it is common practice that cultured zygotes that are not transferred for pregnancy are either frozen for transfer at a later date or simply discarded at the request of the patients. Initial hES cells were derived by immunosurgery of 6-d-old blastocytes by complement-mediated removal of the outer trophectoderm of a blastocyst stage embryo leaving an intact ICM. The ICM was then plated on γ-irradiated or mitomycin C-treated mouse embryonic fibroblasts (MEFs) and cultured in high serum concentrations where ES cell colonies formed after several days in culture *(15,18)*. Since then, a number of other variations have been explored, including hES cell isolation from 8-d-old human blastocysts *(19)* or the feasibility of obtaining ES cell lines from human morule *(20)*.

To date, there are approx 19 "normal" hES cell lines in the National Institutes of Health (NIH) registry available to investigators (*see* http://stemcells.nih.gov/research/registry/). In addition, a repository of hES cell lines with various genetic abnormalities has been established from privately sponsored funding providing an unlimited source of "diseased cells" for research into the primary disturbances of cellular processes in genetic abnormalities. The hES cell lines of genetic disorders were derived from embryos unusable for transfer, deemed to be "mutant" by preimplantation genetic diagnosis; a common practice for IVF. There are 18 hES cell lines with genetic disorders including adrenoleukodystrophy, Duchenne and Becker muscular dystrophy, Fanconi anaemia, complementation group A, fragile-X syndrome, Huntington's disease (three lines), Marfan syndrome, myotonic dystrophy (two lines), neurofibromatosis type I (five lines), and thalassemia (two lines) *(21)*.

Another frontier in hES cell isolation for the study of disease and development was achieved by Hwang et al. *(22)*, who isolated the very first patient-specific, immune-matched hES cell line. It is anticipated that these ground-breaking studies will be important in the advancement of clinical deliberations for stem cell transplantation. Using this approach, Hwang et al. *(22)* generated 11 human embryonic stem cell (hESC) lines by nuclear transfer (NT) of skin cells from patients with disease or injury into donated oocytes. These lines (NT-hESCs) were grown on human feeders from the same NT donor or genetically unrelated individuals and were established at high rates, regardless of NT donor sex or age. NT-hESCs are pluripotent, chromosomally normal, and, most important, are an exact match to the NT patient's DNA. Furthermore, major histocompatibility complex identity showed immunological compatibility, an important milestone for their eventual transplantation and clinical application *(22)*.