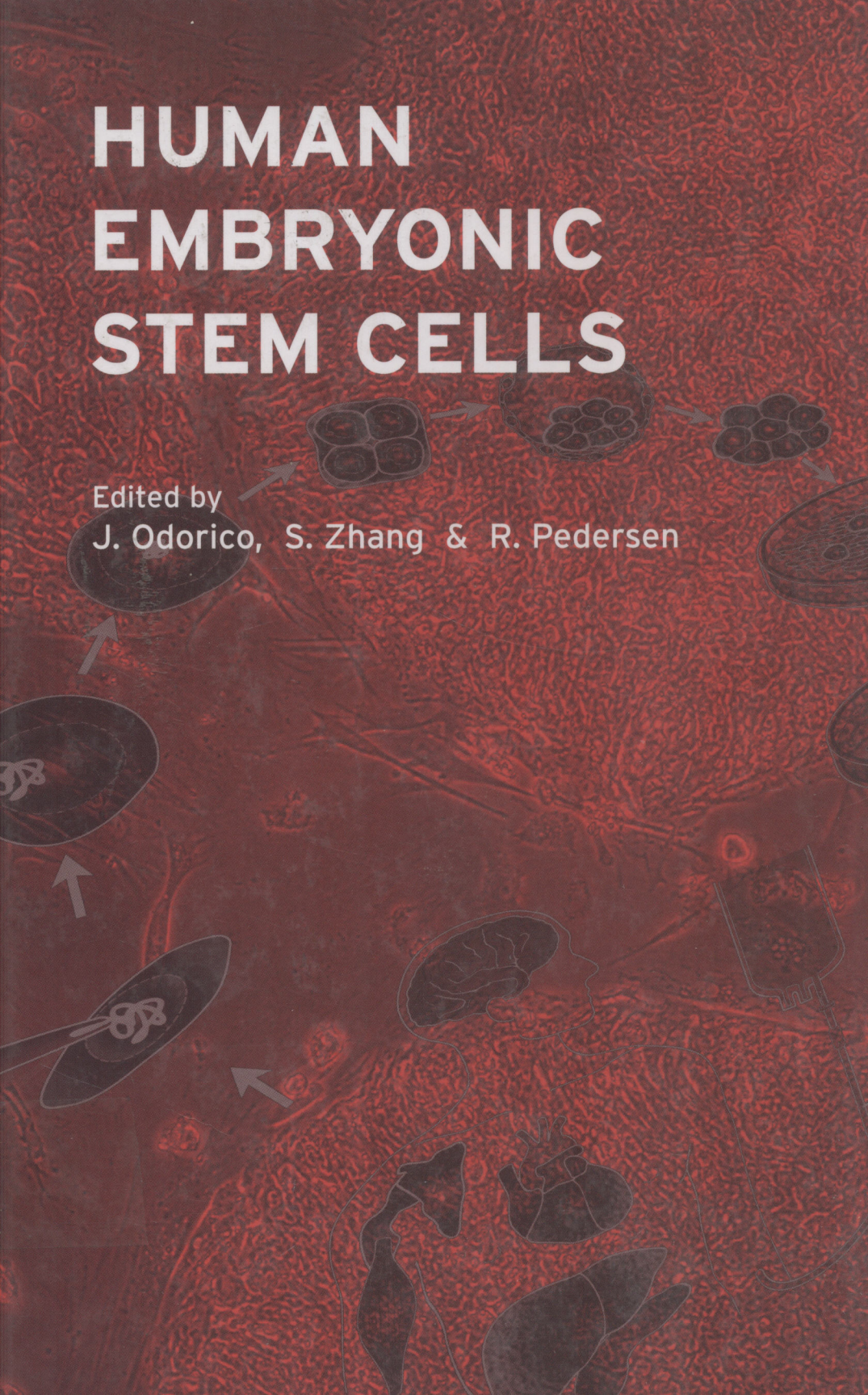
HUMAN EMBRYONIC STEM CELLS



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Abbreviations

2-DGE	two-dimensional gel electrophoresis
ANF	atrial naturetic factor
ANP	atrial natriuretic peptide
AP	alkaline phosphatase
AVE	anterior visceral endoderm
bFGF	basic fibroblast growth factor
bHLH	basic helix-loop-helix
BMP	bone morphogenetic protein
CAFC	cobblestone area-forming cell
cDNA	complementary DNA
CFC	colony-forming cell
CFU-f	colony-forming unit-fibroblastic
CG	chorionic gonadotrophin
cGMP	current Good Manufacturing Practice
CIITA	class II transactivator
CM	conditioned medium
CML	chronic myelogenous leukemia
CMV	cytomegalovirus
COA	Certificate of Analysis
CTB	cytotrophoblast
dsRNA	double-stranded RNA
EB	embryoid body
EBD	embryoid body-derived
EC	embryonal carcinoma
ECM	extracellular matrix
EG	embryonic germ
EGC	embryonic germ cell
EGF	epidermal growth factor

eGFP	enhanced green fluorescent protein
EPC	endothelial progenitor cell
EPL	primitive ectoderm-like
EPLEBs	differentiation of EPL cells as EB
ERRβ	estrogen related receptor beta
ES	embryonic stem
ESC	embryonic stem cell
ESRF	ES cell renewal factor
EST	expressed sequence tag
EVT	extravillous cytotrophoblast
FAA	fumarylacetoacetate
FACS	fluorescence-activated cell sorting
FAH	fumarylacetoacetate hydrolase
FBS	fetal bovine serum
FCFC	fibroblast colony-forming cell
FCS	fetal calf serum
FDA	Food and Drug Administration
FGF	fibroblast growth factor
FGFR	fibroblast growth factor receptor
FKBP	FK-binding protein
FMEA	failure mode and effect analysis
FT-ICR	Fourier transform ion cyclotron resonance
GCSFR	GCSF receptor
GDF5	growth and differentiation factor-5
GDNF	glial cell line-derived neurotrophic factor
GM-CSF	granulocyte/macrophage colony stimulating factor
HCT	hematopoietic cell transplantation
HEF	human embryonic fibroblast
hES	human embryonic stem
HLA	human leukocyte antigens
hMSC	human mesenchymal stem cell
HPLC	high performance liquid chromatography
HPRT	hypoxanthine phosphoribosyltransferase
HSC	hematopoietic stem cell
IBMX	isobutylmethylxanthine
ICAT	isotope coded affinity tag
ICM	inner cell mass
IGF	insulin-like growth factor
IHH	Indian hedgehog
IL	interleukin
IMAC	immobilized metal affinity chromatography
IVF	*in vitro* fertilization
KSR	knockout serum replacement
LIF	leukemia inhibitory factor
LIFR	LIF receptor
LKLF	lung Kruppel-like factor

LPM	lateral plate mesoderm
LTR	long terminal repeat
MACS	magnetic column separation
MALDI-TOF	matrix-assisted laser desorption ionization/time of flight
MAPC	multipotent adult progenitor cell
M-CSF	macrophage colony stimulating factor
MEA	microelectrode array
MEF	murine (mouse) embryonic fibroblast
MEF2C	myocyte enhancer binding factor 2C
MHC	major histocompatibility complex; myosin heavy chain
mHC	minor histocompatibility complex
ML-IC	myeloid-lymphoid initiating cell
MPSS	massively parallel signature sequencing
MS	mass spectrometry
MSC	mesenchymal stem cell
NCAM	neural cell adhesion molecule
NFAT	nuclear factor of activated T cells
NSC	neural stem cell
NT	nuclear transplantation
NTN	neurturin
OCT	octomer-binding transcription factor
PDGF	platelet-derived growth factor
PECAM	platelet/endothelial cell adhesion molecule
PEI	polyethylenimine
PGA	polyglycolic acid
PGC	primordial germ cell
PGD	pre-implantation genetic diagnosis
PhIAT	phosphoprotein isotope-coded affinity tag
PNS	positive negative selection
PSA-NCAM	poly-sialylated neural cell adhesion molecule
PTLD	post-transplant lymphoproliferative disease
PTM	post-translational modification
Q-PCR	quantitative-PCR
RA	retinoic acid
RESC	rat ES-cell like
RF	radiofrequency
RNAi	RNA interference
RT-PCR	reverse transcriptase polymerase chain reaction
SAGE	serial analysis of gene expression
SCF	stem cell factor
SCID	severe combined immunodeficiency
SCNT	somatic cell nuclear transfer
SCX	strong cation exchange
SDIA	stromal cell-derived neural inducing activity
SDS-PAGE	sodium-dodecyl sulfate polyacrylamide gel electrophoresis
siRNA	short inhibitory (or small interfering) RNA

SOM	self-organizing map
SRC	SCID reconstituting cell
SSEA	stage-specific embryonic antigens
STB	syncytiotrophoblast
TDGF1	teratocarcinoma-derived growth factor 1
TGFβ	transforming growth factor beta
TOF	time-of-flight
TPO	thrombopoietin
TSC	trophoblast stem cell
VEGF	vascular endothelial growth factor
vWF	von Willebrand factor

Foreword

The health of human ES cell research

James A. Thomson

The reports of the derivation of human Embryonic Stem (ES) cells (Thomson *et al.*, 1998) and of human embryonic germ (EG) cells (Shamblott *et al.*, 1998) in late 1998 sparked both a wave of scientific enthusiasm and a political controversy that remains incompletely resolved. A partial resolution of that controversy in the United States was made by President George W. Bush when he restricted federal funding to human ES cell lines derived before August 9, 2001. As of this writing (April 8, 2003), only 11 human ES cell lines are listed as available by the National Institutes of Health Embryonic Stem Cell Registry. How damaging to human ES cell research have this and other compromises been over the last four years? A comparison to the early years of mouse ES cell work is useful. Two groups first reported the derivation of mouse ES cells in 1981 (Evans and Kaufman, 1981; Martin, 1981). An informal search of PubMed from July 1981 through November 1985 revealed 14 citations (excluding reviews) involving mouse ES cells. A search covering a similar period (November 1998 through March 2003) revealed 35 non-review articles involving human ES cells. By this superficial measure, at least, human ES cell research appears to be progressing at a reasonable rate.

However, this enumeration of citations ignores significant differences between the initial derivation of mouse and human ES cells. At the time of the initial derivation of mouse ES cells, the mouse experimental embryology community was a small, tightly knit group with only a handful of laboratories having the required expertise to work with mouse embryos or ES cells. To the few members of that small community, the idea of making knock-out mice with ES cells was just a dream, little appreciated by outside researchers. The development of homologous recombination for mouse ES cells and the resulting ability to make knock-out mice spawned an intense interest in mouse ES cells that was no longer restricted to the mouse embryology community. What started with a handful of mouse embryologists now involves most universities and institutes with significant biomedical research programs.

Against this backdrop, the current progress of human ES cell research is somewhat disappointing. Unlike the initial mouse ES cell derivations, there was an almost immediate appreciation that human ES cell research would be broadly important across biomedical research disciplines. This appreciation was due, in part, to the two decades of previous experience by the scientific community with mouse ES cells and to the intense media coverage that made the cells widely known to the scientific community. Yet the rate at which investigators have joined the field has been slower than might have been predicted given the level of interest. There are multiple contributing explanations for this. In the USA, there was no federally funded human ES cell research prior to President Bush's August 9, 2001 announcement restricting federal funding to those ES cell lines derived prior to that date. The initial lack of federal funding and the political uncertainty surrounding the work made investigators hesitant to enter the field. Ignoring the dubious public policy merit of President Bush's compromise, it did have the effect of giving investigators confidence that this work would go forward and be supported. However, the restricted number of existing cell lines created a bottleneck as the investigators involved with the initial derivation scrambled to set up the necessary infrastructure to meet the demand. An even more significant bottleneck was the limited number of groups with the expertise to use human ES cells effectively. Although a number of training courses for human ES cell culture have now been set up to address this need, there remains a significant, inherent time lag between this initial training and the emergence of quality publications. Indeed, the inherent cycle times of graduate and postdoctoral studies are likely to have the most significant long-term effects on the growth curve of the field.

The diversity of investigators contributing to the chapters in this volume suggests that the initial lag phase for the human ES cell field is already coming to an end and that an exponential growth phase is beginning. During the next year or two, it is likely that therapeutically useful human ES cell derivatives will be purified, and that defined culture conditions eliminating the need both for feeder layers and for non-human proteins will be developed. When these events occur, there will be intense pressure for public policy to go beyond President Bush's compromise and for multiple groups to derive new cell lines. Although the political controversy has certainly increased the time lag, the growth curve of the human ES cell field ultimately will be driven primarily by the scientific and medical merit of the cells.

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Preface

The derivation of human embryonic stem cells in 1998 was a landmark discovery that will ultimately allow us to more profoundly comprehend human developmental processes, and which in the future could provide medical therapies for diseases characterized by the failure or destruction of specialized cells. Human embryonic stem cell research crosses many disciplines, including stem cell biology, reproductive biology, molecular biology, immunology, ethics, policy, embryology, neurobiology, oncology, and transplantation. Several chapters in this monograph illustrate the potential for cross-fertilization of ideas and technologies, such as proteomics, gene expression profiling, gene therapy, somatic cell nuclear transfer, prenatal genetic diagnosis, and tissue engineering. It was our goal as editors to stimulate a possible bridging of these disciplines in a fruitful way in future human embryonic stem cell research. In planning this text as a resource for scientists and students with a basic understanding of the principles of cell biology, we felt we should cover the unique biological properties of the cells and present this material in the greater context of other stem cell populations that are present in fetuses and adult organisms. These topics are discussed in the first five chapters. Much has been made of the possibility of generating medically useful cell-based therapies from human embryonic stem cells and the editors felt that an essential part of this text was a discussion of the current status of research towards generating cell therapies for treatment of diseases such as diabetes, Parkinson's disease and heart failure, among others. In Chapter 14 and several chapters that deal with differentiation into specific lineages, experts present recent achievements, current controversies, and future challenges as scientists develop and refine strategies to produce purified populations of functional cells for transplantation. Given the intense public and ethical debate surrounding human embryonic stem cell research, important social, moral, ethical and policy issues as they pertain to research in this field and therapeutic cloning are also presented. However, most would admit we are presently in the 'morula' phase of development of such

therapies, and much work still needs to be done. It is clear that we are only at the end of the beginning, but already human embryonic stem cells have catalyzed the emerging field of regenerative medicine and will likely impact it for many years.

There are two underlying themes of human embryonic stem cell research that deserve mention and which the editors feel do not receive enough attention in the public debate about current research and the merits of these cells. In presenting their respective topics, the editors asked each of the contributors to address both of these themes. First, human embryonic stem cells provide a unique and important window to study early human development. Second, and in turn, a better understanding of developmental mechanisms and how tissues form will help achieve directed differentiation of desired lineages and facilitate effective transplantation therapies (*Figure 1*).

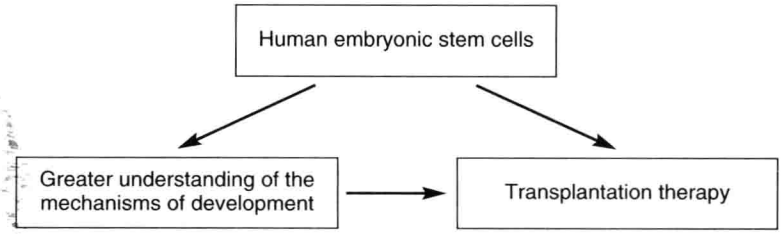

Figure 1: The impact of human embryonic stem cells on biology and medicine.

Although we have learned a great deal about the genetic control of development in many lower species such as frogs, chickens, zebrafish, and mice, we know very little about how and if this knowledge holds true for human development. Despite our understanding of the morphology of organogenesis in human development, there is a major gap in our knowledge of the molecular events that control these processes. Consequently, there is a significant 'species gap' in developmental biology and we are profoundly ignorant about our own, particularly the very earliest stages. For the first time, we have a means to study how the diversity of cell types that make up the human body form emerge, that is, which transcription factors are involved, what other cell types or tissues are inductive, and what signaling pathways regulate these processes in a human context. In areas of study such as placentogenesis, where there are no good small animal models, human embryonic stem cells will provide an insightful supplemental model system. Even if human embryonic stem cells were to fall short of their therapeutic promise, we believe future studies using human embryonic stem cells to study developmental mechanisms will have lasting impact on our understanding of both normal and abnormal human development. In turn, a better understanding of developmental mechanisms and signaling pathways will facilitate the development of effective cell-culture protocols for differentiating human embryonic stem cells into the desired specific cell lineages and will most likely drive the establishment of effective transplantation therapies. The editors hope that the reader will appreciate these two

important and unique aspects of this text and that these themes will stimulate many new and exciting experiments.

We apologize to our many colleagues whose work was not included. Because this is a rapidly moving field, some aspects of the science, technology and policy may have evolved significantly since the chapters were initially written; for this we also apologize to the reader. However, while some details may evolve, we believe that the overarching themes of this book will remain important principles as the science moves forward.

The expertise required to generate this text far exceeds that of its editors. For the superb contributions of each of the authors we owe our sincerest gratitude. Moreover, his book would not have been possible without the assistance of our support staff. We are indebted to Janet Fox, Karen Heim, Kathy Worrall and Liz Cadman. We would also like to express our appreciation for the staff at the Taylor & Francis Group, including Nigel Farrar and Andrew Watts, for their outstanding technical support. Finally, we are grateful for the patience and support of our families during this project.

We hope that this book will provide students and scientists with a greater appreciation of the truly unique properties of human embryonic stem cells. We also hope it will entice new outstanding scientists to enter the field, that it will engender many new experiments among existing stem-cell researchers, and will stimulate focused and redoubled efforts towards generating stem cell-based therapies. At the minimum, we hope that it conveys the important impact this rapidly emerging, but young field can have on our understanding of human development.

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