

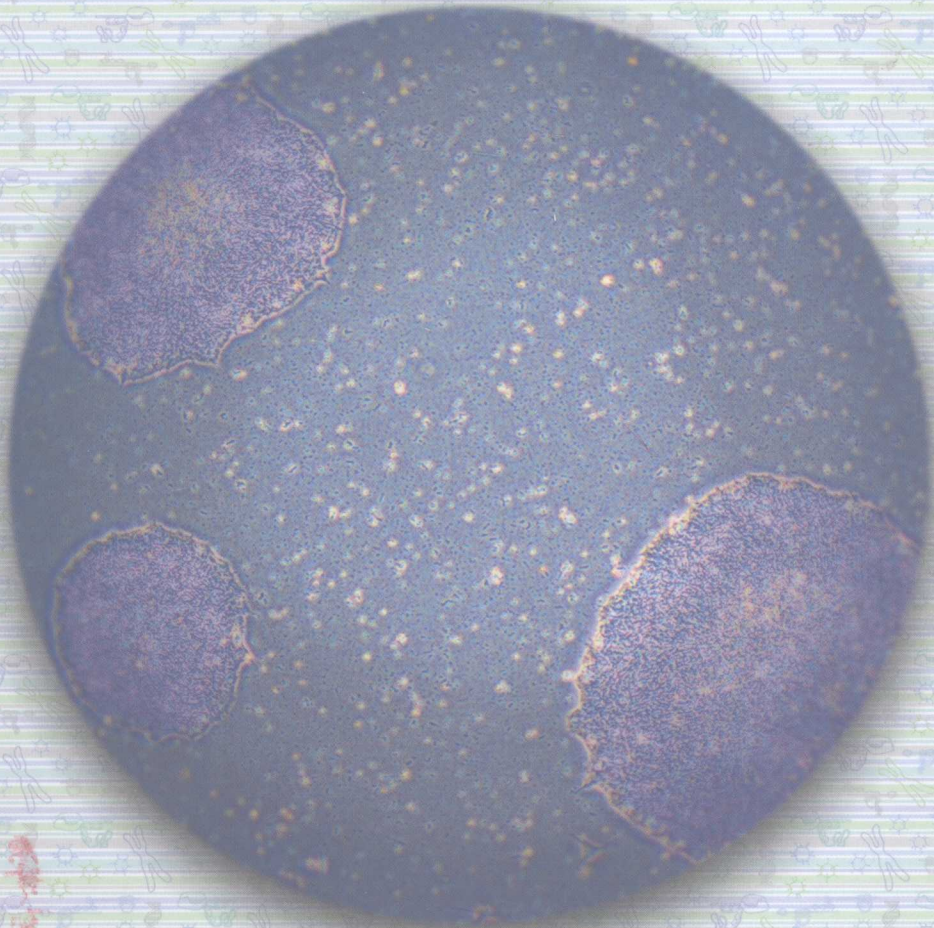
Advances in Molecular Biology and Medicine

Robert A. Meyers

Stem Cells

From Biology to Therapy

Volume 1



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Stem Cells

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Advances in Molecular Biology and Medicine

Edited by
Robert A. Meyers

Volume 1

 **WILEY-BLACKWELL**

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Cover

Image of human iPSCs in feeder-free culture conditions. For more details see figure 1 in chapter 2 "Induced Pluripotent Stem Cells" authored by Kazutoshi Takahashi and Shinya Yamanaka.

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Preface

Five Nobel Laureates are associated with this book: the *Encyclopedia of Molecular Biology and Molecular Medicine* Board members, Sir Martin Evans, who won a Nobel in Physiology or Medicine in 2007 for isolating embryonic stem cells and then growing them in culture; as well as David Baltimore, Gunter Blobel, and Phil Sharp; and now contributing author Shinya Yamanaka, whose 2012 Nobel Prize for Physiology or Medicine was awarded for reprogramming mature cells to become pluripotent stem cells. Professor Yamanaka's chapter on Induced Pluripotent Stem Cells, written for our book, forms a central component, tying together all aspects of stem cells biology and applications.

In his chapter, Professor Yamanaka points out the central issues associated with clinical application of stem cells. "Because pluripotent stem cells can theoretically differentiate into all cell types in the body, applications for cell therapy are expected. However, it is unclear when ES and/or iPS cells would be effective for cell therapy. The most common issue preventing the clinical use of ES and iPS cells is the risk of teratoma formation after transplantation. Residual undifferentiated cells in differentiated cell cultures used for a transplant can cause a teratoma, and should be removed before use. Both effective methods for the removal of undifferentiated cell contamination, such as the use of flow cytometry, and more efficient procedures for differentiation are being developed". Beyond these, there are additional important potential hurdles to clinical applications, including: the need for xeno-free stem cell lines, epigenetic memory and aberrant genetic errors which may be higher for iPS cells as compared with ES cells. All of these factors are covered in detail in our chapters.

The 26 detailed chapters, prepared by leaders in the field, cover the basic biology of stem cells, laboratory methods, stem cells and disease and stem cell therapy approaches and translation to the clinic for treatment of many diseases including Parkinson's disease, spinal cord trauma, diseases of blood cells, and many types of cancer as well as regeneration of cardiac and other muscle tissue. The chapter on "Translating Stem Cells to the Clinic: from modeling disease to cellular products" by Juan Carlos Izpisua Belmonte and his team at the Salk Institute presents the state and future of stem cell clinical applications including 1) "disease in a dish" laboratory substrates providing patient-specific iPS cells which can be employed for disease modeling and drug development; 2) the possibility to generate every desired cell type *in vitro* for restoration of any injury from lost tissue by cell replacement and gene-editing technologies that

efficiently target both and 3) pluripotent cells as well as adult stem cells giving rise to the possibility for gene-correction followed by autologous transplantation which could be employed for the actual cure of monogenic inherited diseases in patients.

Our team hopes that you, the reader, will benefit from our hard work, finding the content useful in your research and educational. We wish to thank our Managing Editor, Sarah Mellor as well as our Executive Editor, Gregor Cicchetti for both their advice and hard work in the course of this project.

Larkspur, California, March 2013

Robert A. Meyers
Editor-in-Chief
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Part I

Basic Biology

1

Epigenetic Regulation in Pluripotent Stem Cells*

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Keywords

Embryonic stem cells (ESCs)

Pluripotent cells derived and cultured from the inner cell mass of blastocysts or from blastomeres of early embryos. These cells are able to proliferate and self-renew indefinitely, and to maintain undifferentiated states under correct culture conditions, while retaining the potential to differentiate into all types of cell in the body.

Induced pluripotent stem cells (iPSCs)

By ectopic expression of a few transcription factors (e.g., Oct4, Sox2, Klf4, and c-Myc), differentiated cells are reprogrammed and give rise to ESC-like cells. The latter are also pluripotent and able to self-renew; hence, they are termed iPS cells (iPSCs).

Totipotency

Cells sufficient to form an entire organism by themselves. Examples are zygotes and few cells in early-cleavage embryos in mammals.

Pluripotency

The developmental potential of a cell to differentiate into all types of cell in the body. The most stringent test for developmental pluripotency is the generation of offspring completely from ESCs/iPSCs by tetraploid embryo complementation, or by four- to eight-cell embryo injection. A less stringent test is the production of germline-competent chimeras by either diploid blastocyst or four- to eight-cell embryo-injection methods.

Reprogramming

An increase in the developmental potency from a differentiated to an undifferentiated stage; also referred to as dedifferentiation in some instances.

Epigenetics

Changes in gene function that are mitotically and/or mitotically inheritable, and that do not entail a change in DNA sequences. Epigenetic information includes changes in gene expression by DNA methylation, microRNAs, histone modifications, histone variants, nucleosome positioning, and higher-order chromatin structure.

DNA methylation

The addition of methyl groups to DNA, mostly at CpG sites, to convert cytosine to 5-methylcytosine. DNA methylation usually represses gene expression.

Histone

Proteins enriched in positively charged amino acid residuals, found in eukaryotic cell nuclei. These proteins package and order the DNA into structural units called nucleosomes.

Nucleosome

The basic unit of chromatin. In a nucleosome, a DNA fragment of 147 bp is wrapped around spools of histone proteins.

Histone modification

Modification in the entire sequence of histones, particularly at the unstructured N-termini ("histone tails"), including acetylation, methylation, ubiquitylation, phosphorylation, and SUMOylation. Histone acetylation or the inhibition of histone deacetylation is generally linked to transcriptional activation.

Imprinting

The allele-specific expression of a small subset of mammalian genes in a parent-of-origin manner (either the paternal or maternal is monoallelically expressed). The establishment of genomic imprinting is controlled mostly by DNA methylation, and also by histone modifications, noncoding RNAs, and specialized chromatin structures. Aberrant imprinting disrupts fetal development, and is associated with genetic diseases, some cancers, and a number of neurological disorders.

X chromosome inactivation

In each mammalian female cell, one of the two X chromosomes is transcriptionally inactivated to compensate any X-linked gene dosage effect between male (XY) and female (XX).

Telomere

Repeated DNA sequences $(TTAGGG)_n$ and associated protein complexes that cap the end of chromosomes to maintain genomic stability. Telomere shortening is associated with cell senescence and organism aging, and also cancer.

Telomerase

An enzyme that specifically adds telomeric repeats *de novo* during each cell division, and is composed of two major components: a telomerase RNA template component (Terc); and Tert, a reverse transcriptase as a catalytic unit. ESCs acquire high telomerase activity to maintain telomere length.

Epigenetic stability is tightly controlled in embryonic stem cells (ESCs) for self-renewal and pluripotency, but is changed during the differentiation of ESCs to various cell lineages. The derivation and culture of ESCs also induce epigenetic alterations, which could have long-term effects on gene expression and the developmental and differentiation potential of ESCs. Developmental and cancer-related genes, and also imprinted genes, are particularly susceptible

to changes in epigenetic remodeling, particularly DNA methylation, microRNA (miRNA), and histone modification. In recognition of the tremendous potential of ESC/induced pluripotent stem cells (iPSCs) in regenerative medicine, the epigenetic instability must be closely monitored when considering human ESCs/iPSCs for therapeutic and technological applications.

1 Introduction

Embryonic stem cells (ESCs) are derived from the inner cell mass (ICM) of blastocysts [1–3]. Under the correct conditions, ESCs are able to proliferate indefinitely. A group of genes is required for ESC self-renewal. These pluripotency-associated genes, which are highly expressed in ESCs, are mostly downregulated upon ESC differentiation [4–6]. Among these genes, three transcription factors – Oct4, Sox2, and Nanog – play pivotal roles in the maintenance of pluripotency [7–10]. These three transcription factors regulate themselves and crossregulate each other, thus, forming a core regulatory circuitry for pluripotency [11]. Moreover, Oct4, Sox2, and Nanog activate many pluripotency-associated genes, while suppressing the expression of genes that encode developmental regulators [11–14].

Importantly, ESCs also have the potential to differentiate into all types of cell in the organism. Typically, ESCs form embryoid bodies (EBs) *in vitro*, which resemble early embryogenesis [1, 2]. Following the subcutaneous injection of ESCs into immunodeficient mice, the cells develop into a benign tumor (a teratoma), which consists of cells from three germ layers [2]. When injected into blastocysts, ESCs contribute to embryonic development and give rise to chimeric animals; subsequently, through germline transmission in chimera, the genetic

information from the ESCs can be passed to the progeny [15]. Most importantly, live pups composed totally of ESCs can be derived by the tetraploid complementation or four- to eight-cell embryo injection [16–18].

These unique properties of ESCs – notably, self-renewal and differentiation potential – are referred to as *pluripotency*. The self-renewal of ESCs can provide an unlimited supply of cells, whereas the differentiation potential of ESCs allows any desired type of cell to be derived. Consequently, ESCs hold great promise for the future development of regenerative medicine.

Epigenetic events are defined as changes in gene function that are mitotically and/or meiotically inheritable and that do not entail a change in DNA sequences. Epigenetic information includes DNA methylation, histone modifications, histone variants, nucleosome positioning, and higher-order chromatin structure. The activities of many enzymes, including DNA methyltransferases (DNMTs), histone demethylases, histone methyltransferases (HMTs), histone deacetylases (HDACs), histone acetyltransferases (HATs), and chromatin-remodeling enzymes, are involved in the regulation of epigenetics [19]. Moreover, as ESCs and differentiated cells share the same genetic materials, the pluripotency of ESCs is mainly attributed to the unique epigenetic regulation within ESCs.

2

DNA Methylation

DNA methylation, which serves as a key epigenetic event in the regulation of gene expression, is in dynamic mode during development. Typically, the paternal genome is actively demethylated in the male pronucleus shortly after fertilization, and this is followed by a passive DNA demethylation of the maternal genome [20]. Global *de novo* methylation increases rapidly in the blastocysts, the earliest stage of differentiation into trophoblast cells, and also in the ICM, from which the ESCs are isolated. The reprogramming of promoter methylation represents one of the key determinants of the epigenetic regulation of pluripotency genes [21]. The methylation of DNA occurs on the cytosine in most cytosine-guanine dinucleotide (CpG) islands in mammalian genomes, and is carried out by various DNMTs. For example, DNMT1 prefers hemimethylated CpGs as a substrate, and maintains the pre-existing DNA methylation pattern during DNA replication. In contrast, DNMT3a and DNMT3b, which are known as *de novo methyltransferases*, prefer unmethylated CpGs as substrate and are responsible for the *de novo* methylation of DNA. The hypermethylation of DNA usually results in repression of gene transcription. Many CpG islands through the genome are hypomethylated and are actively transcribed in undifferentiated ESCs, but subsequently become methylated and silenced during differentiation. Those genes that are repressed in ESCs but required for later differentiation are marked by bivalent H3K4me3 and H3K27me3 domains, that render them poised for activation [22, 23]. Approximately one-third of genes that are not marked by histone H3 lysine 4 trimethylation (H3K4me3) or H3K27me3,

but are mostly repressed in ESCs, are marked by DNA methylation, complementary to histone modifications [21, 22, 24]. The DNA methylation patterns are better correlated with histone methylation patterns than with the underlying genome sequence context. DNA methylation and histone modification pathways may be interdependent, with any crosstalk being mediated by biochemical interactions between the SET domain histone methyltransferases and the DNMTs [25]. Moreover, the polycomb group (PcG) protein Enhancer of Zeste homolog 2 (EZH2) is a histone methyltransferase that is associated with transcriptional repression, interacts (within the context of the Polycomb repressive complexes (PRC) 2 and 3) with DNMTs, and also exerts a direct control over DNA methylation [26].

Undifferentiated ESCs express high levels of the *de novo* DNA methyltransferases DNMT3a and DNMT3b, which may repress differentiation-related genes, thereby maintaining the ESCs in undifferentiated states. Both DNMT3a and DNMT3b are directly regulated by the core pluripotency transcription factors Oct4, Sox2, Nanog, and Tcf3. In addition, they are also indirectly regulated by the miR-290 cluster that represses retinoblastoma-like 2 (Rbl2) [27], which in turn downregulates DNMT3a and DNMT3b [28]. The inactivation of both DNMT3a and DNMT3b in mouse ESCs was shown to cause a progressive loss of methylation in various repetitive sequences and single-copy genes. Typically, DNMT3a and 3b are both stably associated with each other in ESCs [29], with the two enzymes interacting to methylate the promoters of Oct4 and Nanog genes in differentiating ESCs. The methylation of key regulatory genes Oct4 and Nanog