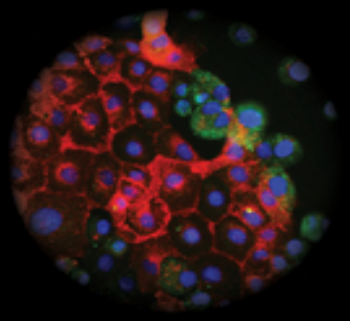
PRINCIPLES OF STEM CELL BIOLOGY AND CANCER

Future Applications and Therapeutics



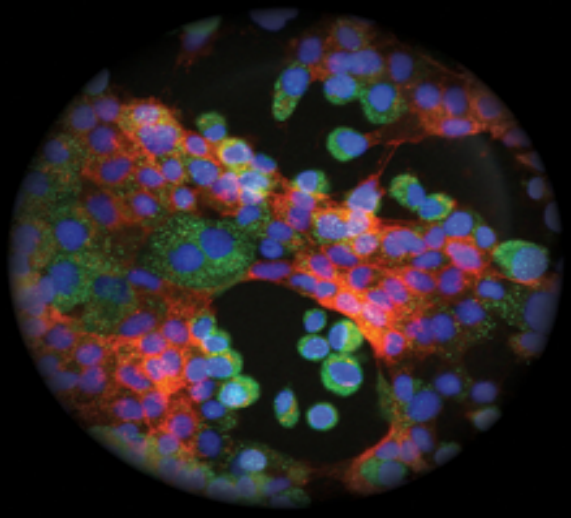


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Table of Contents

List of Illustrations

List of Tables

Principles of Stem Cell Biology and Cancer: Future Applications and Therapeutics

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Preface

Stem cells are a population of cells capable of differentiating into diverse specialized cell types, or of undergoing self-renewal to produce more stem cells. There are two types of stem cells: embryonic stem cells, isolated from the blastocyst, and adult stem cells, found in different tissues of the body. These cells are essential in generating different cell lineages and thus maintaining the structural and functional integrity of tissues and organs. The decision whether to self-renew or to differentiate is tightly regulated and requires a strict control of cell division and cell-cycle exit. This level of control involves key molecules implicated in cell-cycle regulation, as well as several critical growth factors and cytokines. The balance between self-renewal and differentiation can be the target of oncogenic events, leading to cell transformation and the emergence of 'cancer stem cells', which are thought to be subpopulations of cancer cells responsible for tumour progression, development of metastases, tumour dormancy, cancer relapse and resistance to chemotherapy.

In recent years, the stem cell field has become a subject of extensive research, with many groups focusing on isolating and identifying cancer stem cell populations. This effort relies on identifying molecules expressed preferentially by cancer stem cells, with the aim of developing cancer therapies targeting these specific molecules in this cancer population without affecting the pool of normal healthy stem cells. Although some progress has been made, developing efficient therapies targeting cancer stem cells remains one of the important challenges facing the growing stem cell research community.

This book will provide a detailed introduction to stem cell biology. Part I focuses on the characterization of stem cells,

the progress made towards their identification and their future therapeutic applications. Part II focuses on cancer stem cells and their role in cancer development, progression and chemoresistance, and presents an overview of recent progress in therapies targeting cancer stem cells. We believe that this book will be unique in providing compiled information about the link between stem cell biology and cancer. The contributing authors are renowned experts in the field and will provide a timely book of high quality, outlining the current progress in and exciting future possibilities for stem cell research.

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Part I

Stem Cells

Chapter 1

Isolation and Characterization of Human Embryonic Stem Cells and Future Applications in Tissue Engineering Therapies

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1.1 Derivation of human embryonic stem cells from the ICM

1.1.1 Early development of the ICM: the cells of origin for hESCs

The mammalian zygote (fertilized ovum) is defined as being totipotent, as it is capable of developing into a new offspring and the placenta required for full gestation. The zygote initially undergoes cleavage-stage cell division, forming cells (early blastomeres) that remain totipotent. With further development to the preimplantation blastocyst stage, a primary cell differentiation results in outside trophectoderm cells (TE) and an inside aggregate of inner cell mass (ICM) cells. The TE forms placental tissue and membranes, while the ICM forms the foetus and extra-embryonic membranes. Therefore, ICM cells are defined as being pluripotent, forming all cells of the developing offspring other than the complete placenta (unless genetically manipulated). Embryonic stem cells (ESCs) are

derived *in vitro* from ICM cells, which adapt to specific conducive conditions that enable indefinite cell proliferation (self-renewal) without further differentiation and thereby confer a pluripotent capacity. This *in vitro* pluripotent state is due principally to the induction and maintenance of expression of key ‘gate-keeper’ genes, including Oct4, Nanog and Sox2, which then regulate one another (Silva & Smith, 2008). The capacity for self-renewal is sustained by high telomerase activity, which protects chromosome telomeres from degradation during mitosis (Blasco, 2007).

Mammalian ESCs were first derived in the mouse (mESC) (Evans and Kaufman, 1981; Martin, 1981). When mESCs are integrated into an embryo and returned to a recipient, they can contribute to all cell lineages, including germ cells. Their utility soon became invaluable for many transgenic procedures. Successful derivation of human (hESC) lines was reported by Thomson *et al*. (1998), who essentially followed the same procedure as used for the mouse. ICMs isolated from preimplantation human blastocysts were plated on to mitotically inactivated mouse embryonic feeders in culture medium with basic fibroblast growth factor (bFGF) and foetal calf serum (FCS). This culture medium was also supplemented with leukaemia inhibitory factor (LIF), a cytokine necessary to maintain mESCs (Smith *et al*., 1988), although (as is now known) not necessary for standard hESC derivation. Human ESCs display (or lose on differentiation) plasma membrane expression of stage-specific embryonic antigens (SSEAs) that correlate with the preimplantation morphological development of human embryos (Henderson *et al*., 2002) and form teratomas (benign tumours) in immune-deficient mice that can contain cell phenotypes from the three major cell lineages (endoderm, mesoderm and ectoderm), as well as trophoblast. The differentiation of trophoblast cells

indicates that hESCs are not entirely equivalent to mESCs, as usually defined, but align with slightly later LIF-independent mouse epiblast pluripotent stem cells, which have the propensity to differentiate to trophoblast *in vitro* (Brons *et al.*, 2007).

1.1.2 Derivation of hESCs

Success in the derivation of hESCs depends in part on the quality of the human embryos used (usually blastocysts from days 5 to 8), although cell lines have been generated from morphologically poor embryos. Numerous hESC lines have been derived (Figure 1.1) from normal, aneuploid and mutant embryos from patients undergoing treatment for assisted conception (IVF, ICSI) or preimplantation genetic diagnosis (PGD) who consent to donate them for stem cell research. Some of these cell lines have been extensively characterized and compared, enabling international standards to be established (Adewumi *et al.*, 2007).

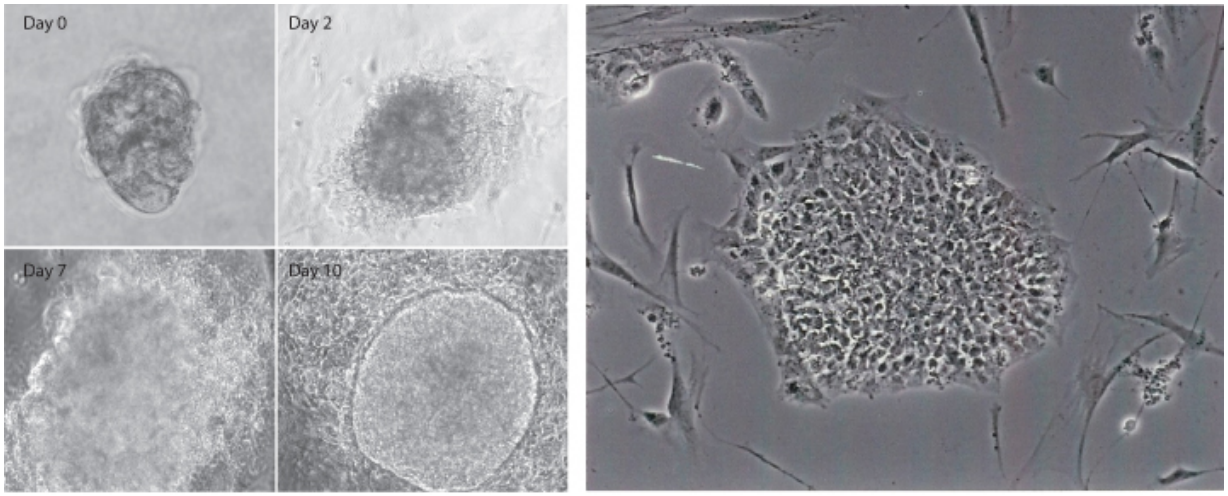

Figure 1.1 (A) Outgrowth of hESCs over 10 days of culture from ICM. In this instance, a clearly defined colony was observed by 10 days, which was mechanically passaged. (B) hESC line Shef1 plated on ECM.

1.1.2.1 Evolution to a more efficient and better-defined derivation method: drivers and technologies

Over the last 15 years, continuous improvements have been made in the process of deriving and maintaining hESC lines. The emphasis initially was on improving efficiency and consistency in the stem cell laboratory. But as hESC lines have become readily available for research in many countries, the focus has changed to devising methods for deriving clinical-grade cell lines that comply with health care regulatory authorities (e.g. Federal Drug Administration, FDA; European Medicines Agency, EMA), which can be used as starting materials for potential cell-therapy trials. Xeno-free methods (free of nonhuman animal components) are preferable as they minimize the risk of cross-species contamination with adventitious agents. An important early improvement was the replacement of FCS with a serum extract (knockout serum replacement, KOSR) to reduce hESC differentiation. This modification also minimized batch variation (inherent in FCS) between culture media, and allowed consistency in the proliferation of the cells after passaging (transfer of cells to a new culture vessel). Subsequently, more defined culture media (xeno-free) have been devised, which, in combination with a variety of extracellular matrix (ECM) compositions, facilitate the proliferation and passage of pluripotent hESCs in the absence of feeder cells (mouse or human), which otherwise remain an ill-defined and inconsistent component of the cell culture. Manipulation of the embryo has also changed over time. Initially, the ICM was isolated according to mouse protocols using enzymatic (protease) removal of the zona pellucida (ECM surrounding blastocyst) and immunosurgical lysis of TE with antitrophoblast antibody to prevent TE culture outgrowth from inhibiting early ESC proliferation. However, xeno-free methods using laser-assisted removal of the zona and

plating of the intact blastocyst or the ICM on to a defined matrix (e.g. laminin 521) with a defined culture medium is the method of choice, leading to successful feeder/xeno-free cell line production in ~20–40% of attempts with good-quality human embryos (Hasegawa *et al.*, 2010). With further improvements to the cell adhesion matrix and cell medium, the efficiency of hESC line derivation is likely to increase further, although the quality of the embryo used to develop ICM cells remains a crucial factor.

Another important consideration is the genetic character and stability of the hESC line. Generally, most hESC outgrowths and initial cell lines derived from unselected embryos (i.e. not PGD selected) are determined to be karyotypically normal within the precision of the chromosomal analysis. However, hESCs acquire genetic mutations in culture, which may endow them with a selective cell culture advantage, so that mutated cells predominate (Baker *et al.*, 2007). Since derivation and ESC passage represent key stress events for ESC cultures, minimization of selective pressure on cells at these stages may help to maintain their normal karyotype. For example, the proliferation of cells by mechanical division of hESC colonies into smaller aggregates may be preferable to enzymatic disaggregation to single cells, which will initiate apoptotic stress pathways unless inhibited from doing so by a chemical inhibitor (i.e. ROCK inhibitor).

1.1.3 Regulation of embryo research and hESC derivation

The destruction of the preimplantation human embryo in order to derive hESC lines has prompted fierce ethical debate in many countries, especially on religious grounds, which to some extent remains unresolved and irresolvable. The result is the implementation of policies of ethical oversight, regulation and permission for hESC research,

which vary from country to country, and even within a country (the United States). In the United Kingdom, early introduction of laws related to human embryo research and the formation of a regulatory body (Human Fertilisation of Embryology Authority, HFEA) provided a framework (and important public confidence) for continuation of hESC research. Clinical-grade hESCs must meet compliance with conditions set by the EMA and overseen in the United Kingdom by the Human Tissue Authority. In the United States, the FDA and National Institutes of Health (NIH) undertake this responsibility. Since the development of cell therapies using pluripotent stem cells is novel, it remains to be determined exactly how regulatory authorities will implement conditions of compliance.

The induction of pluripotency in mouse and human somatic cells in 2006–07 using retroviral vectors to introduce four genes to reprogramme the genome (*Oct4*, *Sox2*, *Klf4*, and *c-Myc*) and enable the derivation of induced pluripotent stem cells (iPSCs) (Takahashi *et al.*, 2007) radically changed the landscape of human pluripotent stem cell (hPSC) research (Yamanaka, 2012). This technology not only provides a potential route for the creation of patient-specific stem cell lines for use in cell therapies but also makes pluripotent cell lines available to many more laboratories, with seemingly fewer ethical bottlenecks. However, hESCs remain the current gold standard as their cellular reprogramming events are those that are normally evoked in the early embryo, rather than artificially induced, and they are therefore less likely to be subject to aberrant epigenetic effects on their gene function. Moreover, ethical issues related to obtaining informed consent from donors to use tissue samples to derive iPSCs still persist. Progress in the use of hESCs (or iPSCs) for therapy will depend on whether robust protocols for their expansion and differentiation to a precise and economic manufacturing