Engineering Stem Cell Fate

A hurdle in the development of many cell-based therapies is securing a reliable source of cells. The choice of cell source is dictated primarily by the specific application. For example, tissue-engineered skin constructs incorporating allogeneic keratinocytes and fibroblasts, used to treat burns and diabetic foot ulcers, have proved to be quite suitable due to the temporary nature of the graft, which is replaced by host cells. Most host tissues, however, are not endowed with robust regenerative capacity, and thus, the search for cells with regenerative potential has become a driving concern for many investigators.

The isolation of embryonic stem cells from mice (mESC) and humans (hESC) has galvanized the field of regenerative medicine in this respect. ESCs are the foundation from which all germ and somatic cells are derived, and many investigators are searching for mechanisms within the field of developmental biology to drive ESC fate to specific cell lineages.

ESCs are obtained from the inner cell mass of a blastocyst-stage embryo. Two approaches (currently unsupported by federal grants) are used to obtain human embryos and derive hESCs from them: 1) cryopreserved cleavage- and blastocyst-stage embryos, produced by in vitro fertilization (IVF) for clinical purposes; and 2) cloned embryos created by nuclear transplantation, produced for research purposes.

In the first approach, embryos are thawed and cultured to the blastocyst stage. The zona pellucida is chemically removed, and the inner cell mass is isolated by immunosurgery and cultured in vitro.

The second approach provides a way to derive autologous ESCs, and thus, its use may circumvent immunologic issues associated with transplantation. This method creates embryos by transplanting the nuclei of a somatic cell (termed somatic cell nuclear transplantation, SCNT) into an enucleated meiosis II oocyte, which is then activated to divide. Although cytoplasmic factors within the oocyte do reprogram the somatic cell genome to a primordial state, this epigenetic reprogramming is incomplete, and cloned organisms raised to term can develop major clinical complications.

An alternate modality is therapeutic cloning, whereby karyotypically and phenotypically normal ESC lines are derived from the cloned embryo. In a reduction to practice, George Daley and Rudolph Jaenisch et al. used SCNT to derive mESCs from a mouse model depleted in lymphocytes due to a genetic deficiency. They used gene therapy to correct for the mutated gene ex vivo and subsequently differentiated the cells along a lymphocyte lineage. When these cells were reintroduced back into the same mouse, a significant lymphocyte population was observed that was capable of stable expression of the gene of interest.

This study underscores the potential of SCNT in cell transplantation, though technical limitations still remain. SCNT is cumbersome, expensive, and not amenable to high-throughput generation of ESC lines, which limits scale-up of this technology. Also, cellular limitations of SCNT include the need for an oocyte source independent of IVF clinic use, and the need for determining success of SCNT using post-mitotic cell nuclei.

Recent studies have begun to address these limitations. For example, Hans Scholer et al. showed that oocytes could actually be derived from mESCs. In addition, Rudolph Jaenisch et al. observed that fertile mouse clones could be created from SCNT of terminally differentiated cell nuclei. Notwithstanding these accomplishments, more in-depth studies are needed to understand the mechanisms by which the oocyte can reprogram a somatic cell, which may ultimately allow in vitro manipulation of a somatic cell by synthetic means.

The potential of ESCs to affect clinical medicine relates to their basic properties of self-renewal and pluripotency. *Self-renewal* is the capacity of a cell to proliferate indefinitely while remaining in an undifferentiated state.

Certain aspects of self-renewal are not conserved from mouse to human, including: phenotypic markers of self-renewing cells; signal transduction pathways involved in self-renewal; and methods to maintain a self-renewing population. For example, the action of a single cytokine, leukemia inhibitory factor (LIF), can maintain mESCs in an undifferentiated state, yet hESCs are not responsive to LIF and require a mouse embryonic feeder layer to self-renew. Much focus has been directed toward revealing the mechanisms of self-renewal using genomic and proteomic methods in order to provide an hESC line devoid of xenogenic contaminants for clinical use.

Pluripotency refers to the ability of ESCs to reconstitute all three

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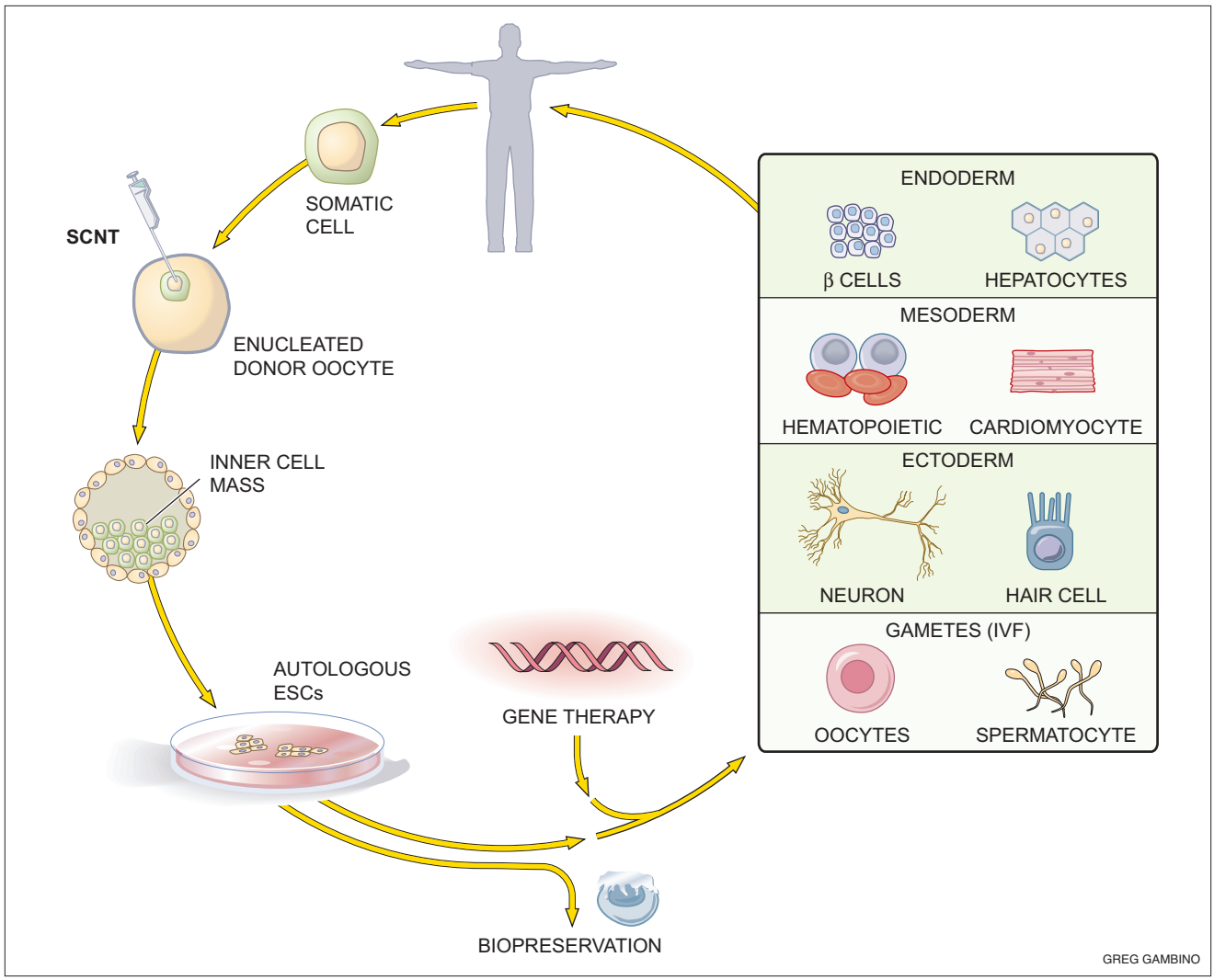

ESC replacement therapy using SCNT (somatic cell nuclear transplantation). The nucleus of a somatic cell biopsied from a patient is injected into an enucleated donor oocyte, and the oocyte is then activated to divide. The inner cell mass, containing autologous ESCs, is isolated, subcultured, and preserved, if needed. A therapeutic gene can be introduced into these cells to correct for a genetic deficiency. ESCs then can be differentiated into a specific cell type in vitro and reintroduced back into the patient.

germ layers (endoderm, ectoderm, and mesoderm) in vivo. To form one distinct cell type, an ESC makes many lineage-specific decisions that materialize in differential expression of certain genes and their proteins, coupled with higher order functional and morphologic changes.

These lineage-specific decisions are influenced by cell interactions with its microenvironment, namely surrounding cells, secreted factors, and extracellular matrix proteins. Investigators have sought to mimic the stimuli encountered in natural cytogenesis in vitro in order to direct ESC lineage choice to adult cell types that have limited, if any, regenerative capacity in vivo. To this end, the traditional application of ESC research is replacement therapy for monocellular deficiencies that are not reliably supported by an alternative cell source, such as an adult stem/progenitor cell, though many other applications are being explored (see table).

Below, we briefly discuss several examples of the isolation of therapeutically relevant cell lineages from ESC cultures, transplantation studies in certain animal models, and novel culture conditions to engineer ESC-derived cells.

Neurodegenerative Disease

The pathogenesis of neurodegenerative diseases such as Parkinson's, Alzheimer's, and Huntington's diseases involve irreversible injury to certain functional neurons. While neural stem/progenitor cells exist, they have not shown a significant ability to repopulate all types of neural tissue. Due to this regener-

ative limitation, ESCs may be a practical alternative to derive specialized subtypes (approx. 200) of nervous system cells for replacement.

Lorenz Studer et al. induced differentiation of mESCs and SCNT-derived mESCs into a wide range of neuroectodermal cells. Using stromal feeder cell lines (used primarily for hematopoietic stem cell culture), they developed protocols to selectively generate dopaminergic, serotonergic, cholinergic, and GABAergic neurons as well as glial cells. The dopaminergic neurons were shown to alleviate a parkinsonian condition when grafted into mice with 6-hydroxy-dopamine-induced lesions.

In another study, the same laboratory derived midbrain dopaminergic neurons from hESCs. With the aid of medium supplementation of known morphogens and transcription factors encountered during embryogenesis, these neurons sequentially expressed mRNA transcripts indicative of the midbrain, showed action potential sensitivity to tetrodotoxin, and released dopamine from tyrosinehydroxylase-positive synaptic terminals.

In an effort to coax stem cell fate using small synthetic molecules, Peter Schultz et al. first reported the use of pharmaceutical screens to induce neuronal differentiation of mESCs. A kinase-directed combinatorial library led to the discovery of an optimal drug candidate that induced neurogenesis as indicated by a green fluorescent protein-reporting mESC line.

Cardiovascular Disease

A subset of cardiovascular diseases can be attributed to cardiomyocyte injury and death. Because there is no precursor cell to replenish cardiomyocyte loss, an infarction, if large, can lead to primary pump failure. Timothy Kamp et al. observed that hESCs can differentiate into distinct classes (atrial, ventral, and nodal) of human embryonic cardiac muscle. Each derived subtype mirrored the electrophysiologic and pharmacologic responses that are indicative of their respective anatomic region.

Lior Gepstein and Joseph Itskovitz-Eldor et al. demonstrated that hESC-derived cardiomyocytes can be functionally integrated into host tissue. Isolated cardiomyocyte grafts from hESC cultures formed structural and electromechanical connections with cultured rat cardiomyocytes. Also, in vivo integration of hESC-derived cardiomyocytes was observed by imaging techniques, and the treatment proved efficacious in pacing a porcine heart with a complete atrioventricular block.

Larry Shears II et al. reported a selection method for ESC-derived cardiomyocytes by exposing cultures to nitric oxide (NO) or by gene transfer of NO synthase. NO tended to induce differentiation of cardiomyocyte-committed cells while causing apoptosis in undifferentiated cells, thereby enriching a population of interest by means of both positive and negative selection.

Diabetes

Autoimmunity to insulin-producing β cells of the pancreas results in type I diabetes. The "Edmonton protocol," which successfully combined cell transplantation with steroid-sparing immunosuppression to restore normoglycemia, has revolutionized treatment of the disease. However, the scarcity of donor tissue remains an obstacle.

APPLICATIONS OF ESC RESEARCH

Clinical medicine	• Derivation of functional cells for replacement and/or combination therapy
	• Mammalian cell vector for gene therapy
	• Preservation of ESCs without loss of viable, pluripotent cells after reanimation
Pharmaceutical	• Embryonic toxicity tests
	• Drug candidate screening
Basic science	• Platform to study developmental biology
	• In vitro model of congenital disease (e.g., trisomy 21)
	• Model of human tumor physiology
	• Cellular aging and senescence studies

Bernat Soria et al. first showed that insulin-secreting cells could be derived from mESCs and normalize glycemia in streptozotocin-induced mice. Employing an insulin gene trapping technique to selectively isolate transfected mESC clones, the group observed in vitro insulin responses to various secretagogues and in vivo modulation of high glucose levels in a diabetic animal model.

Ron McKay et al. devised a multistaged differentiation protocol that included isolation and expansion of progenitor populations in different selection media. The derived cells formed islet-like structures and expressed temporal phenotypes similar to those seen in endocrine pancreas development.

In an attempt to drive endocrine pancreatic differentiation rather than its developmentally similar neural counterpart in ESC cultures, Seung Kim et al. treated ESCs with phosphoinositide 3-kinase (PI3K) inhibitor, which has been shown to increase endocrine cell mass in human fetal pancreas while preventing neurite outgrowths from neuroendocrine cells. ESCs treated with PI3K inhibitors resembled pancreatic β cells phenotypically and positively regulated glucose homeostasis and survival of streptozotocin-induced diabetic mice.

ESC research is in its infancy, and many challenges remain. The basic problem—i.e., how can cell phenotype be controlled and optimized to induce lineage-specific function?—will require extensive study and input from many fields. The solutions likely will involve new approaches and tools to direct, enhance, or possibly circumvent normal embryogenesis. The multidisciplinary approach that tissue engineers employ, integrating developmental/stem cell biology information with engineering and materials science principles, can help drive ESC research from proof-of-principle laboratory studies to the next generation of clinical medicine.

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