Types of Stem Cells

Embryonic stem (ES) cells do not actually come directly from embryos—instead, they are cultured counterparts of certain cells from embryos. Nearly as powerful as the fertilized ovum, ES cells can, depending on their biochemical surroundings, follow several trajectories: remain quiescent, “self-renew” to produce more of themselves, or differentiate (specialize) as any of the 260+ types of cells that comprise a human body (Figure 1.1). ES cells are termed pluripotent, and not totipotent, because unlike a fertilized ovum, they can produce only body cells, and not also the membranes that support the embryo. Even though ES cells are not natural-occurring, understanding their activities and fates is shedding light on our physiology and pathology, and thereby suggesting many exciting new clinical applications, especially for degenerative diseases.

Figure 1.1. Pathways to Cell Specialization

2.2. Tools and Technologies in Context

Experiments and protocols use naturally existing stem cells or derive them from ES cells or exposure to lysates or introduced factors. Similar tools are required to obtain, amplify, identify, modify, and, if used therapeutically, deliver these cells. This section contrasts two examples to illustrate the reagents and other support materials in context: working with ES cells with a focus on basic research; and using umbilical cord stem cells to treat a lung disease in a novel way.

Culturing ES Cells

ES cells must be characterized as such, and then either maintained or stimulated to differentiate down specific lineage pathways. Steps for culturing iPS cells once they have been transfected with factors and directing differentiation are similar.

Characterizing ES Cells

Since the first gene expression studies to define “stemness” in 2002, reviewed in Chapter 1, ES cell characterization has shifted from the cellular to the molecular (Table 2.2). In addition to providing more precise information, molecular descriptions overcome certain limitations of cell culture. These include the tendency of the cells to aggregate, which blocks cell surface antigens, and having to destroy cells to detect enzymes and transcription factors.

Table 2.2. Some Characteristics of hES Cells

<table>
<thead>
<tr>
<th>hES cells…</th>
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</thead>
<tbody>
<tr>
<td>• Possess immortality</td>
</tr>
<tr>
<td>• Have normal karyotypes</td>
</tr>
<tr>
<td>• Are clonogenic</td>
</tr>
<tr>
<td>• Derive from the inner cell mass</td>
</tr>
<tr>
<td>• Give rise to three primordial germ layers</td>
</tr>
<tr>
<td>• Integrate into the germline</td>
</tr>
<tr>
<td>• Express Oct4</td>
</tr>
<tr>
<td>• Differentiate in response to altered culture conditions</td>
</tr>
<tr>
<td>• Require feeder cells and serum, or feeder cells and serum-free medium and bFGF</td>
</tr>
<tr>
<td>• Form flat aggregates or embryoid bodies</td>
</tr>
<tr>
<td>• Are in S phase of the cell cycle</td>
</tr>
</tbody>
</table>

Source: Insight Pharma Reports
Applications

muscle, smooth muscle, and endothelium when in the environment of a healthy heart. Control hearts also had cells with the stem/progenitor markers assessed in the study (c-kit, MDR1, and Sca-1), indicating that the healthy heart also has these cells.

Meanwhile, studies in animal models supported the Anversa work, suggesting that stem cells from bone marrow and elsewhere might heal hearts. Anecdotal human reports began to make headlines. A case in point: Sixteen–year-old Dmitri Bonnville, who was working a construction job when a nail gun harpooned him in the chest. After the nail was removed at a hospital in his Michigan town, Bonnville suffered cardiac arrest and heart damage. With consent, physicians gave the young man a colony stimulating factor to increase release of stem cells from the bone marrow, then collected the cells and injected them near his heart. Bonnville improved steadily for months. The story apparently vanished after his parents sued the hospital, but for botching the initial nail removal, not the stem cell treatment.

The Bonnville and other case reports posed questions that are gradually being answered in pilot studies and larger trials. What is the mechanism behind improvement in the months following cardiac cell therapies? Do bone marrow stem cells naturally home to the heart? While there, do they replace damaged tissue by transdifferentiating into a cardiac lineage, fuse with resident cells, or exert paracrine effects on resident cells, rather than stimulating regeneration? How much of the recovery is due to the treatment and how much is due to natural healing? Does a single cardiac stem cell give rise to all three cell types, or are there different types of stem cells at play?

Recent reports address some of these questions. They also reflect the continuing diversity of approaches to treating heart disease with stem and progenitor cells.

• **Pluripotency:** Anversa’s group introduced cardiac progenitor cells activated with insulin-like growth factor 1 and hepatocyte growth factor into coronary arteries near infarcts in rats. The progenitors engrafted and divided, giving rise to endothelium, smooth muscle, and cardiomyocytes.

• **Re-establishing pacemaking:** Cardiomyocytes derived from ES cells (ESC-CM) are electrically active, but in an immature, less controlled way than their counterparts in a heart, which
“Brent was growing adult striatal tissue and saw something that everyone else just threw away. So he called Weiss over, and said, look, these are balls of neurons and glia,” recalled Dennis Steindler, PhD, executive director of the McKnight Brain Institute, who recently recruited Reynolds. “We put EGF, which was thought to be protective, on the cells, and they proliferated like crazy,” said Reynolds. A colleague suggested that they clone individual cells, and the cells took on the persona of stem cells. That wasn’t surprising, given the fetal source of the tissue, so Reynolds looked at adult tissue, sampling striatum that included ventricle tissue where, unknown at the time, stem cells lie in the ependymal layer. “Had we looked at cortical cultures, nothing would have worked. So it was serendipity,” he said.

The cells in non-adherent culture formed balls, and Reynolds and Weiss figured out how to passage them. They struggled with a name. “We thought about calling them nerve balls but thankfully we came up with neurospheres,” Reynolds said. (See Figure 4.1.)

**Figure 4.1. Tumor Neurosphere**

This neurosphere, consisting of human neural stem cells, derives from glioblastoma multiforme tumor cells.

*Source: Brent Reynolds, PhD, McKnight Brain Institute, University of Florida*
Participants

George Daley, MD, PhD, is past president of ISSCR and an associate in medicine at Children’s Hospital Boston. Dr. Daley moderated the discussions from which these comments were taken.

Shinya Yamanaka, MD, PhD is senior investigator, Gladstone Institute of Cardiovascular Disease and the L.K. Whittier Foundation Investigator in stem cell biology and professor of anatomy at the University of California, San Francisco and director, Center for iPS Cell Research and Application and professor, Institute for Frontier Medical Sciences, Kyoto University, Japan. He led the team that first derived iPS cells in mice and then humans.

Kathrin Plath, PhD, an assistant professor at UCLA, was a post-doctoral researcher in Rudolf Jaenisch’s lab at the Whitehead Institute.

Rudolf Jaenisch, MD, is a member of the Whitehead Institute and a professor of biology at MIT (Cambridge, MA).

Junying Yu, PhD, is an assistant scientist in James Thomson’s lab at the University of Wisconsin in Madison. The Thomson lab was the first to derive hES cells, in 1998.

Sir Ian Wilmut, PhD, is director of the Centre for Regenerative Medicine at the Queen’s Medical Research Institute, University of Edinburgh. Dr. Wilmut was head of the team that used SCNT to clone sheep, including Dolly, at the Roslin Institute.

1. When the derivation of human iPS cells was announced in late 2007, the press and certain politicians stated or implied that these cells would supplant human ES cells, which are controversial because of their reliance on cells from embryos to establish cell lines. What are scientific views on the relationship between hES cells and iPS cells from patients? That is, how have human ES cells been important, and how will they continue to be so?

Dr. Daley: I’ve spoken out on behalf of ISSCR that our policy is that the extreme excitement around iPS cells does not in any way obviate the value to science of ES cells, including new ES cell lines generated by nuclear transfer.
Chapter 6
SELECTED COMPANY PROFILES

It is difficult to profile only a handful of the 700+ companies that work with stem cells or supply the tools of the trade. Such companies define themselves in various ways, from the rather vague “stem cell research and development” and “a stem cell engineering company” to the prescient “personalized stem cell therapies for tissue regeneration.” Basically companies that supply cells can be classified by cell source and application in basic research, drug discovery and development, or therapeutics. For now, adult stem cells dominate the landscape, especially for regenerative medicine, but it is the hES and iPS cells that will likely lead the way in drug testing applications. The companies profiled here were selected for their diversity.

6.1. Cellartis

Location: Arrid Wallgrens Backe 20, SE-413 46 Goteborg, Sweden and Maclagan House, 1 Wurzburg Ct., Dundee DD2 1FB UK

Phone Number/Fax Number/Web site: Sweden: 4631 758 0900/4631 758 09 10/www.cellartis.com UK: 44(0)1382 56 99 70/44(0)1382 56 82 42

Founding Information: Founded 2001

Selected Management: Mats Lundwall, CEO; Johan Hyllner, PhD, CSO, COO; Kristina Runeberg, vice president business development

Number of Employees: 50

Financial Information: Privately held by a Scandinavian syndicate of life science venture investors.