Special News Feature

Banking on (Pluri) Potential

Photo credit: Jeff Miller, University of Wisconsin-Madison.

Wrestling with issues of provenance, pluripotency, and performance, stem cell banks do more than just freeze and ship cell vials.

For all the political, scientific, and ethical hand-wringing that accompanied the 21 infamous embryonic stem cell lines approved in 2001 for use in federally funded research by then-President George W. Bush, the fact remains that those cells were available to the scientific community. Researchers can thank a half-dozen facilities scattered across the United States, Israel, Sweden, and Singapore for initially keeping and distributing these cells, and then—starting in 2005—the National Institutes of Health (NIH)–funded National Stem Cell Bank (NCSB).

Like their financial counterparts, cell banks are repositories, centralized facilities that, in this case, accept, validate, store, expand, and distribute scientifically useful cell lines to the larger research community. Some are public, others are private; some serve local interests, while others support the broader community. All wrestle with fundamental issues of logistics, infrastructure, and workflow.

But stem cell banks must clear additional hurdles, too, especially if they deal in human embryonic stem cells (hESCs).

“We are not talking about blood or hair,” says Rosario Isasi, an attorney and research associate at McGill University in Montreal, of hESCs; “we are talking about something all humans find a morally sensitive area.” Isasi has studied stem cell governance for six years and recently

coauthored a review on the subject (1). She says the most pressing ethical concern is “provenance:” ESC donors must be able to ensure that the cells they bank, like biological “blood diamonds,” were not unethically obtained.

On the technical side, hESCs are perhaps the trickiest and most temperamental cells biologists will ever grow, the cellular embodiment of the phrase “more art than science.” “It’s almost [as if]... it’s dependent on the phase of the moon when you start the cells as to whether they are going to grow well or not,” says Glyn Stacey, director of the UK Stem Cell Bank. Genetically unstable and prone to differentiate, hESCs must be maintained manually (i.e., without automation), and it can take months of hands-on experience before researchers become adept at working with them.

Testing, testing, testing

The list of tests to which stem cell banks subject their hESC lines can be exhaustive. In 2009, the International Stem Cell Banking Initiative produced a “consensus guidance” document including “a recommended minimum set of criteria for release of hESC cell banks.” (2) The recommendations included 10 different assays, from ensuring that the line matches its parental line, to tests for pluripotency, cell antigen expression, and genetic stability.

At the Wisconsin International Stem Cell (WISC) Bank, which took on the NSCB’s duties when the national bank’s federal funding ended in February 2010, the testing regimen includes 27 separate assays including post-thaw viability, mycoplasma testing, gene expression profiling on NimbleGen microarrays, karyotyping, comparative genome hybridization, and flow cytometry, to name a few. The cells are also screened for human and animal pathogens such as HIV, bovine viral diarrhea virus, and porcine adenovirus.

“This testing is extensive—almost too extensive,” says Erik Forsberg, executive director of the WiCell Research Institute, which oversees the WISC Bank. Forsberg

Single cardiomyocyte derived from human iPS cells and labeled with antibodies showing the organization of the contractile proteins. Blue, nuclei; green, α -actinin; red, myosin light chain 2A. Courtesy of Jianhua Zhang.

Timothy Kamp says iPS cells can differ in their ability to form heart cells.

estimates that each of the NSCB lines costs nearly \$60,000 to test. WISC Bank hESCs that are not part of the NSCB are tested less exhaustively, as are iPS cells, according to Forsberg; for instance, these lines are not assayed for bovine, porcine, and murine viruses. Nevertheless, these lines still cost about \$3000 each to test.

Compare this to the treatment traditional cell lines receive. At the American Type Culture Collection (ATCC), cell lines such as NIH 3T3 are tested for viability, contamination, morphology, and species identification: intraspecies identification of human lines using short tandem repeat analysis, and interspecies identification using amplification of the cytochrome C oxidase I gene. Some cells (including the ATCC's few stem cell lines) receive extra testing, too; PC-12 cells, for instance, are tested for their ability to form neurites in response to growth factor, whereas 3T3-L1 cells are tested for their ability to form adipocytes.

And cell lines are not just tested once; most cell banks adopt a three-tiered system to maintain their lines. Initially, a submitted sample is grown and then frozen to make a token (or premaster) stock. Next, a token aliquot is expanded to make a seed (or master) stock, from which the distribution lots—that is, the frozen vials that are shipped out to researchers—are made.

ATCC tests its cells at every stage, according to Brian Douglass, cell biology product manager, at a cost of “thousands of dollars” per line. If the cells fail just one test, he adds, the whole lot is discarded.

WISC Bank tests lots at both the master and distribution stages, including “characterization studies” at the distribution point that assess the cells’ surface antigens, gene expression patterns, and chromosomal stability every five passages, says Forsberg. “There’s a real propensity for [the cells] to develop abnormal chromosomal and gene expression profiles. We fail cell line lots on a regular basis.”

What’s your potential?

Tim Kamp, director of the Stem Cell and Regenerative Medicine Center at the University of Wisconsin Medical School, has acquired hESCs as well as iPS cells from the WISC Bank. His goal is to assess those cells’ ability to form different types of heart cells for research applications and potential clinical uses. iPS cells, he says, “are not all equivalent in terms of ability to form heart cells. Some are better than others.”

But hESCs and iPS cells must be able to do more than form cardiac tissue; as pluripotent progenitors, they must have the potential to form all three germ layers of the body. The question for cell banks is, how best to *quantify* that potential in a production environment?

At the Coriell Institute for Medical Research in Camden, NJ, which began accepting iPS cells in late 2009, pluripotency testing can last up to six months, and is “pretty labor-intensive,” according to Margaret Keller, director of the Coriell Stem Cell Biobank. “The characterization that’s involved is beyond anything we have done before.”

The regimen includes flow cytometric assessment of surface markers SSEA4 (a pluripotency marker) and SSEA1 (a differentiation marker); real-time PCR analysis of reprogramming and pluripotency markers (e.g., Nanog, Oct4, and Sox2); embryoid body formation; and directed differentiation experiments. In a directed differentiation assay, cells are supplied with growth factors to induce formation of cardiac, pancreatic, or neural cells (representing mesoderm, endoderm, and ectoderm, respectively). The cells’ ability to form those cell types is a measure—albeit only a qualitative one—of the line’s pluripotency.

The final validation step, teratoma formation, involves injecting iPS cells into immunocompromised mice, where the cells form a benign tumor (a teratoma) whose composition represents the various germ layers.

But teratoma tests are problematic, says Martin Pera, director of the Broad Center for Regenerative Medicine and Stem Cell Research at the University of Southern California, because the differentiation is very random. “You might see muscle in one bit, and nerve cells in another bit, and it’s impossible to tell because it is chaotic and jumbled up,” he says. While murine ESCs can be genetically tagged and inserted into developing embryos, implanted into a foster mother, and allowed to develop to term, such an assay obviously cannot be done with human cells. With teratomas, it is also impossible to assess the efficiency of differentiation based on the cells researchers do observe. “There may be bits missing, or you just may not be

Passaging embryonic stem cells. Courtesy of Jeff Miller, University of Wisconsin-Madison.

seeing them,” Pera says. “It is a very qualitative assay.”

Pera is an advisor to the International Stem Cell Initiative (ISCI), which in 2007 analyzed the cell surface expression of 59 hESC lines, identifying 17 surface markers that could be used for comparison (3). According to Peter Andrews—the Arthur Jackson Professor of Biomedical Science at the University of Sheffield, UK—who coordinated that first ISCI project, the group is preparing for ISCI-3, which will attempt to identify a standard set of methods for evaluating the pluripotency of a given cell line, especially iPS cells.

The issue isn’t identifying new methods, Andrews explains, but is one of consensus. “What we are looking at in ISCI-3 is if the community can agree on a number of simple, robust, and quantifiable protocols to assess whether a given stem cell is able to differentiate in a given direction.”

Ethical considerations

Technical challenges aren’t the only issues facing hESC banks. In the recently released bestseller, *The Immortal Life of Henrietta Lacks*, author Rebecca Skloot delves into the ethical issues surrounding the development of HeLa cells, which were derived without consent from a woman dying of cervical cancer. Embryonic stem cells demand even greater scrutiny, says Isasi, since the embryos from which they derive could theoretically grow into human beings.

“For some people this could be a conversation stopper,” says Isasi. “If the derivation was not ethically done or legally done, the consensus is your cell line quote-unquote is tainted.”

The key issue is provenance, and according to Isasi, it encompasses three key criteria: informed consent, monetary compensation, and donor confidentiality. The UK Stem Cell Bank’s Code of Practice for the use of Human Stem Cell Lines, for instance, requires each gamete provider in an embryo donation to acknowledge, among other things, that they understand that cell lines derived from these donations may be used for treatment purposes in the future, and that they will not benefit financially from any commercial or patented application of their donation.

Wisconsin’s Kamp, who has deposited hESCs to the WISC Bank, says it wasn’t difficult to prove provenance for the lines he banked, because they were genetically modified derivatives of already approved lines. “There’s no doubt there is some time

commitment to deposit these lines,” he says. “But it’s a necessary evil to be sure, because like any bank, you don’t want tainted currency, and you don’t want currency that’s counterfeit.”

Even iPS cells, which are thought to be ethically less incendiary, are not without controversy, Isasi adds. Because the technology is both complicated and rapidly evolving, how thoroughly can potential donors be informed as to researchers’ intent?

Several efforts are underway to standardize best practices and simplify ethical issues. The International Society for Stem Cell Research, for instance, is planning a cell registry with associated proof-of-provenance to cut down on red tape.

“They will do their own sort of validation and then they will provide a certificate saying we accept that we can prove ethical provenance of that line,” Isasi explains. “So then you take that certificate and you can go to your [institutional review board] and apply for funding. Or, some small stem cell banks can use that as an assurance that the information that is provided by a researcher is valid and correct.”

Meanwhile, ISCI-3’s development of pluripotency evaluation standards, which started in March 2010, could bear fruit by next spring, says Andrews.

“We are trying to demonstrate that there are methods available to grow the cells more conveniently and in a more mechanized way that would not also affect the properties of the cells,” says Stacey, whose UK Stem Cell Bank is part of ISCI. “When we can do that, our life as a bank will change dramatically, because the whole banking process will be significantly easier.”

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