Stem Cells: Hype vs Reality, or Political Science?

Curt Civin, MD
Assoc Dean for Research
Director, Center for Stem Cell Biology
& Regenerative Medicine
University of Maryland School of Medicine
Bermuda (March, 2009)
Learning Objectives

1. Define *stem cell*
2. Describe *adult vs embryonic* stem cells
3. Describe how *ES cell lines* are made
4. Consider the *future* medical potential of stem cell research
5. Consider the *current* medical limits of stem cells
Sometimes I wonder if there's more to life than unlocking the mysteries of the universe.
What is a stem cell?

- A "stem cell" is a *single* cell that has the ability to self renew and differentiate *extensively*, generating both:
  1. all (totipotent) or most (pluripotent or many [multipotent]) types of specialized cells, and
  2. new stem cells with identical potential (self-renewal).

- So, the defining features of a stem cell are that it can differentiate and self-renew extensively.
A “(lympho)hematopoietic stem cell” is a *single* cell that has

- **self-renewal capacity**: can generate *many* new HSCs, and

- **multipotent differentiation capacity**: can generate all of the cells of the blood-immune system (≥11 specialized types of cells).

The defining features of a hematopoietic stem cell (HSC) are that it can generate new HSCs, as well as the entire blood/immune system.
Paradigms of “adult” stem cells, hematopoietic stem cells form only (mainly) blood cells

The Development of Blood Cells

From Undifferentiated Stem Cells to Mature Blood Cells

- Self-Renewal
- Pluripotency

Development of all 11 lineages of mature blood and immune cells from a single, pluripotent stem-progenitor cell; and probably endothelial cells, as well
Blood-forming stem cell self-renewal is limited, *in vitro*

- **Regulators:**
  - SCF
  - TPO
  - FL
  - IL-1
  - IL-6
  - G-CSF
  - IL-3
  - IL-11
  - GM-CSF
  - G-CSF
  - IL-4
  - M-CSF
  - IL-2
  - IL-6

Lack of self-renewal capacity is a limitation of all types of “adult stem cells,” in addition to their limited ability to differentiate to multiple tissue types.
Blood-forming stem cell transplant: Icon

The CD34 antibody developed in my Hopkins lab has been used in bone marrow transplants for thousands of patients.

And research studies on purified CD34 blood stem cells have resulted in thousands of basic scientific and applied medical discoveries.
Identification & purification of hematopoietic stem cells: Financial disclosure

The Johns Hopkins University holds patents on CD34 monoclonal antibodies and related inventions.

- Dr. Civin is entitled to a share of the sales royalty received by the University under licensing agreements between the University, Becton Dickinson Corporation and Baxter HealthCare Corporation.

- These arrangements are being managed by the University in accordance with its conflict of interest policies.

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Federal Appeals Court Backs Hopkins Patent

Victory in Intellectual Property Rights Lawsuit Sets Key Precedent for University Researchers

By Brooke Southall

Daily Record Business Writer

Johns Hopkins University has won a final court victory in a key battle over intellectual property rights that it believes will keep private firms from stealing its patents, and those of other universities.

The U.S. Court of Appeals in Washington upheld a July 1997 decision by a federal district court in Wilmington, Del., that ordered Bothell, Washington-based biotechnology firm CellPro Inc. to pay $7 million in damages to JHU and its licensees.

The upheld appeal was a landmark victory, according to Curt Civin, inventor of the patented stem cells and professor of oncology and pediatrics at Johns Hopkins University.

"The principle was very, very important to universities," he said. "Had the case gone the other way, companies would have had had no incentive to deal with universities. I think the patent system would have just gone away."

Injunction upheld

Civin added that JHU alone has hundreds of inventions every year.

SEE CELLPRO PAGE 6A
What is a human embryonic stem cell?

Requires oocyte (egg) harvest:

- Hormone treatment of woman (pseudo-pregnancy)
- “Minor” surgical procedure with anesthesia

- Medical definition of “minor” surgery = surgery that happens to someone else
- Ethical issues in egg donors (coercive circumstances, payment)
What is a human embryonic stem cell?

- The "embryo" is the stage between the ovum (egg) and the fetus (beyond 2 months) in prenatal development.
- When does human life begin?
- Embryonic stem cells are made from cells of the "inner cell mass" of the blastula.
From zygote to blastula: the early stages of human development

- When does human life begin?
- Development of neural streak
  - first embryonic structure
  - day 14 gestation

Blastocyst: 5-7 days

Uterine implantation: rarely successful
Pluripotent embryonic stem cells can self-renew indefinitely and differentiate into all types of human cells.
Human embryonic stem cells in tissue culture on mouse feeder cells

- They don’t look human!
**In Vitro** Differentiation of Human ES Cells

- Embryoid Bodies
- Neural Cells
- BFU
- CFU-GM
- Epithelial
Human endoderm from HESCs


What is an embryonic stem cell line?

- alk phosphatase$^+$
- SSEA-4$^+$
- GFP$^+$ colony after lentiviral transduction

Embryoid body containing hot pink primitive erythroid cells (embryonic + fetal hemoglobin)
Derivation of human embryonic stem cell lines

When does human life begin?
Should the embryo have all the rights of a human being?

Nature 413:13, 2001
“Therapeutic Cloning”: generation of embryonic stem cells after somatic cell nuclear transfer (SCNT).

Snyder, N Engl J Med 2006

“Reproductive Cloning”: “I cloned a guy in Reno. How ‘bout yourself?”

“I cloned a guy in Reno. How ‘bout yourself?”

New Yorker 10/8/01.
How long are stem cells grown *in vitro* before they are used?

- Elias Zambidis, MD/PhD, getting some “Presidential” human embryonic stem cells that are growing in the tissue culture incubator.

- The cell lines grow immortally (indefinitely) in tissue culture.
Why do I use **embryonic** stem cells in current studies of blood cell development?

- Embryonic stem cells can self-renew in the lab for years, and make enough hematopoietic stem cells to transplant many individuals (Hem stem cell transplant as a means to tolerize patients for transplants of stem cells to form organs such as kidney, pancreas, etc)

- Using embryonic stem cells, we can investigate very early steps in blood (and leukemia) development that occur even before a woman could know she was pregnant

- Embryonic stem cells can form a variety of cell types beyond blood

- Embryonic stem cells as models to understand cancer stem cells
Why not use adult stem cells?

- Adult stem cells are less “plastic” (less versatile in ability to differentiate, less pluripotent).
  - For example, blood-forming stem cells (from bone marrow, blood, or neonatal placental/umbilical cord blood) form blood cells (predominantly).
  - In contrast, embryonic stem cells can form all the cell types of the body.

- Adult stem cells have limited self-renewal
  - But we hope that studies of ES cells will lead us to ways to teach adult stem cells to self-renew more expensively.
What diseases can be helped by stem cell research?

- Estimated over 70 diseases: Diabetes, Parkinsons, heart failure, Alzheimers, cancers, …
- 128 million Americans
- Including a member of 50% of families
Wishful thinking?

Human Therapeutic Cloning

Patient

Somatic cell biopsy

Enucleated donor oocyte

Nuclear transfer

Embryonic stem cells

Pancreatic islet cells

Hematopoietic cells

Cardiomyocytes

Neurons

Hepatocytes

Immunologically Compatible Transplant

Stem cells rebuild MI-damaged tissue in mice

Managing patients with heart attacks could dramatically change if the preclinical results translate into the clinic.

By Henry W. Slager
Managing Editor
SAN FRANCISCO -- When injected directly into heart tissue, hematopoietic bone marrow cells could help repair tissue damaged as a result of a myocardial infarction (MI), according to investigators at the National Institutes of Health in Bethesda, Md.

Because heart tissue lacks the ability to repair itself after damage, the transplant of stem cells could repair myocardium and help prevent further Mls in some patients, Donald Orlic, MD, told attendees at the plenary session of the 62nd Annual Meeting of the American Society of Hematology. The technique has not been used in patients, but experiments in mice have proven very successful.

"Stem cells could differentiate into cardiac tissue that would then engraft," Orlic and his team produced infants in the left ventricle of normal adult female mice by orchectomy of the left common artery (LCA).

In earlier studies, Orlic and his team used Langerin-expressing progenitor cells from the bone marrow of adult transgenic mice.

They injected between 2.1 x 10^8 Langerin-expressing progenitor cells, which expressed enhanced green fluorescent protein (EGFP), into the healthy myocardium adjacent to the infarcted region three to five hours after LCA occlusion. Six to 12 days after transplantation, the hearts were removed and prepared for immunohistological analysis.

Restored function

"Mice which had received transplanted bone marrow showed an improvement in function that approached function value in normal controls," Orlic said, explaining that control mice did not have induced heart failure.

Further analysis of the EGFP-labeled cells revealed they were positive for cardiac specific myosin and alpha-actinin, a protein specific for both cardiac and skeletal myocytes.

In a cell proliferation assay, BrdU was injected daily for four days. Approximately 28% of the EGFP-positive myocytes were positive for BrdU, a protein expressed in cycling cells.

"These findings suggest a high level of cell cycling in the donor cell population as early as six days post-transplant," he said.

Orlic also noted that the observed Ki-67-positive endothelial cells in microvessels in the infarcted myocardium.

Curing Sickle Cell Anemia in a Humanized Mouse Model

First case of tumor development in a human following (fetal = “adult”) stem cell therapy, though similar findings have been made when embryonic stem cells were injected into rats.

The immunologic deficits of the boy’s A-T may itself have allowed the allogeneic tumors to develop.
House Bill 810 passed by Senate by vetoed by Pres. Bush

- **Sen Brownback bill**: Broad ban on human embryonic stem cell research
- **Sen Hatch, Specter, Kennedy, Feinstein bill**: Federal funding for human ES research, including SCNT expts allowed, up to 14 days (neural streak appearance: precedents in England, California)
- **Rep Castle House bill**: Allow new ES cell lines to be made from leftover frozen IVF embryos with informed consent of donors
- **Neither side has a veto-proof majority**

Thus, current Presidential policy prevailed: Human ES cell research was not prohibited, but US fed govt funding was restricted to studies using human ES cell lines made before Aug 9, 2001 (NIH funds ~90% of biomed research in US). On March 9 2009, President Obama announced that he will lift the guidelines, and Congress may also act.
Opponents of stem cell research

- Tactic #1: link the debate on stem cell research to irreconcilable differences on:
  - when life begins
  - the morality of abortion
  - general discomfort with scientific progress
We’re not going to clone any kind of people!

And nobody in my lab is going to clone elephants.
Tactic #2: “Adult” stem cells will do the job

- The current scientific merits and limitations of adult vs embryonic stem cells have been mentioned. Most expert scientists agree that research on both adult and embryonic stem cells should be allowed and encouraged. The findings and uses may be complementary, something like the CAT scan and the MRI.
Plastic stem cells, even from adult stem cells (mice)

Donor (male) epithelial cells 11 months after BMT

Krause, *Cell* 2001

*Science* 290:1674, 2000
What are iPS cells? Mature cells such as adult human skin fibroblasts that have been “reprogrammed” to pluripotent ES like state and behavior

Retroviral infection with OCT4/SOX2/MYC/KLF4 or OCT4, SOX2, NANOG, and LIN28

Mature human fibroblasts (from foreskin or adult skin) were engineered with Nanog-GFP or Oct4-Neo reporter retro- or lenti-vectors

Transduced pluripotent stem cells were green or Neo-resistant

Reprogrammed fibroblasts could be propagated (maintaining undifferentiated markers) for >20 passages or induced to undergo multilineage differentiation (to form all 3 germ layers both in embryoid body assays in vitro and in teratoma assays in immunodeficient NOD/SCID mice)
On controversial national issues:

- States can be "laboratories of democracy"
- States as opportunities for multiple experiments
  - Bills currently under discussion in ~20 states
- Then cull best laws and federalize
  - Uniform, federal policies, procedures eventually needed for efficacy in science
Forbids human reproductive cloning

- Imposes criminal penalties
- Maryland now one of a handful of states, including California, where reproductive cloning is illegal

Funds stem cell research

- Human adult and embryonic stem cell research
- SCNT (therapeutic cloning) will not be funded by MSCRF, but remains legal (a compromise)
- Legislature must reevaluate and can renew the law in 5 yrs
- No defined yearly budget (another compromise), but widely perceived target = $20-25M/yr
- In Maryland law, all funding depends on the yearly gubernatorial budget proposal, then legislative approval or reduction
What *kind* of stem cell research can be supported by MSCRF?

Many advocates wanted to fund *only* human embryonic stem cell research. *Rationale:*

- HESCs have greatest pluripotentiality and self-renewal capacity
- Funding HESC research would precisely fill the current US Federal funding gap

Others wanted to fund *all* stem cell research. *Rationale:*

- Fund the best stem cell science (All stem cell research is currently underfunded)
- Adult or embryonic stem cells may be optimal for given applications

*Compromise* made to end a filibuster in the State Senate and secure Governor’s support: Fund *all* kinds of *human* stem cell research
Huge conceptual change, increasing funding

State of MD is successfully playing the new role of providing sustaining funding for stem cell research

- $15M in grant awards announced May 2007
- $23M appropriated for 2008 and recently awarded
- $19M appropriated for 2009
- Governor O’Malley favors $20-25M/yr for the remainder of the first 5 yrs, if the overall MD budget can handle it
How to make grant awards?: Peer review, minimizing conflicts and politics

Scientific **peer review** by panels of **expert stem cell researchers from other states**

Since MSCRF monies must be spent in MD, this simple mechanism greatly reduced expert reviewer conflicts.

**Second tier oversight** by an appointed **Commission of Marylanders.** **Considerations:**

- **Ethics**
- **Geographical distribution**
- **Breadth of research portfolio**
  - All kinds of stem cells
  - Basic, translational, clinical research
  - New and established investigators

- 86 applications were submitted in year 1 (2007); total requests >$80M.
- 147 grant applications were submitted, requesting $85M for the $18M available in this year’s MSCRF/TEDCO budget.
An example:

- My own lab’s current research on hematopoietic stem cells seeks to understand the regulation of stem cell function, at the fundamental gene-microRNA level:

- However, our motivations for seeking this understanding are translational:
  - to manipulate stem cell regulation transiently, and thereby to expand these stem cells ex vivo and then to direct their differentiation for much better transplantation and transfusion therapies, and
  - to down-regulate the stem cells that sustain leukemias and other cancers
What’s happening?: We have successfully cultured very early blood-forming “blast” cells from the “Presidential” H1 and H9 hESC lines

Colonies of undifferentiated human embryonic stem cells

Day 2: Differentiating human embryonic stem cells

1-2 weeks later: Many differentiating human embryonic stem progeny

2 weeks later still: Colonies of functional blood cells

Zambidis, Blood 2005
What else is new?: We have discovered a new set of controlling molecules ("microRNAs") which restrain the growth and development of blood-forming stem cells.
What slows our research?

- **Old tools for embryonic stem cell research:**
  - We can use only “version 1.0” (“Presidential”) human embryonic stem cells (no antibiotics, slow growth, clinically limiting)

- **Insufficient funding for embryonic stem cell research:**
  - Slow development of preliminary data → delays in grant funding

- **Strange barrier to scientific interactions:**
  - Prohibitions prevent federally-funded researcher from teaming up to bring in ideas and expertise, as we usually do in our open culture of discovery and translational science
What is the promise of our studies?

- Matching hematopoietic stem cell transplants (BMT) available for everyone
- Hematopoietic stem cell transplants to prevent rejection of transplanted organs, such as kidney, pancreas, etc (or hESC-derived organ-forming cells)
- Understanding that will lead to new medicines to treat and prevent diseases (Whole-world treatments and preventions instead of expensive, first-world transplants)
What will be the impact of human embryonic stem cells?

- **Knowledge**
  - Understand normal and abnormal human tissues

- **Drug discovery & testing, environmental toxicology**
  - Test human tissues in the test tube, before human clinical trials

- **Clinical transplantation**
  - Caveat: Complexity of human diseases

- *Political* bottlenecks slowed the entire impact of stem cell research, not just clinical transplantation (the *icon*)
Cancers likely originate in stem cells

➢ Their longevity and (self-)renewal capacity should make stem(-progenitor) cells susceptible to the acquisition of both initiating and secondary oncogenic mutations (in the same cell):
  ❖ Stem(-progenitor) cells persist long enough to accumulate the multiple oncogenic hits necessary for cancer development
  ❖ Stem(-progenitor) cells are already programmed to generate huge clones of identical or similar progeny cells

➢ These cancer stem cells persist and maintain the fully-evolved cancers
➢ Are cancer stem cells vulnerable to inhibition of canonical stem cell signaling?
“To him who devotes his life to science, … his cup of joy is full when the results of his studies immediately find practical applications”

-- Louis Pasteur

**Worst jobs in science: #16**

1. FLATUS ODOR JUDGE
2. DYSENTERY STOOL-SAMPLE ANALYZER
3. BARNYARD MASTURBATOR
4. BRAZIL MOSQUITO RESEARCHER
5. HOT-ZONE SUPERINTENDENT
6. ISOLATION CHAMBER TESTER
7. FISTULA FEEDER
8. PRISON RAPE RESEARCHER
9. CARCASS CLEANER
10. POSTDOC
11. METRIC SYSTEM ADVOCATE
12. CORPSE-FLOWER GROWER
13. ENDANGERED SPECIES ECOLOGIST
14. ASTRONAUT
15. FISH COUNTER
16. **US STEM CELL RESEARCHER**
17. PLANETARY PROTECTION OFFICER
18. FUSION RESEARCHER

William Speed Weed

*Poplar Science* Sept 2003
Every year, in Europe and America, hundreds of thousands of embryos created for couples by in vitro fertilization (IVF) are thrown away. Every one contains embryonic stem cells, the amazingly potent cells that can grow up to be anything—from liver to blood to bone to skin. "From a pure-science point of view, embryonic stem cells are more powerful than the genome project," says Johns Hopkins pediatric oncologist Curt Civin. "They could tell us what each and every gene actually does. And they could be used to cure cancers, Parkinson's disease, diabetes." You name it. But by and large, American researchers must stop there—at the hopeful act of recognizing the potential. Their ability to study actual stem cells is hobbled by the federal regulation triggered in 2001 by President Bush's famously faux-Solomonic—tear the baby in half!—decision to limit the cells a federally funded researcher can study to those coming from the 78 cell lines cultured prior to the date of the regulation. In practice, though, only 11 approved lines have been made available to researchers. It's like handing an oceanographer a cup of salt water and saying, "Study only this."

In contrast, the sensible British have got it right, says Civin. Under strict regulation, and culling from IVF throwaways, doctors are allowed to create their own embryonic stem cell lines. "We're going to be trumped," says Civin. "I'd like to figure out everything there is about blood stem cells, but in all, the discovery is going to be slower, and as an American, I'm not going to be a part of it."
Vision and charge:
Create a stem cell research initiative that will foster a broad range of interdisciplinary research designed
• to understand stem cell biology

• and, at the same time, to directly affect human health and disease

Developing novel diagnostic methods, treatments, and/or preventions for major human diseases will be a key, immediate part of each major project.
Lamason et al. SLC24A5, a putative cation exchanger, affects pigmentation in zebrafish and humans. *Science* 2005; 310:1782 (and cover)

Deep relevance of science to society:

- Black to white skin because of a single change in 1 gene.
- This is due to a mutational change in 1 base of DNA, an A to a G, like a typo. But we’ve made this typo into so too much more than it means in nature.
Is this science relevant to you?
72-year-old man with worsening cough and difficulty breathing