Scientists grow two new stem cell lines in animal cell-free culture

January 2 2006

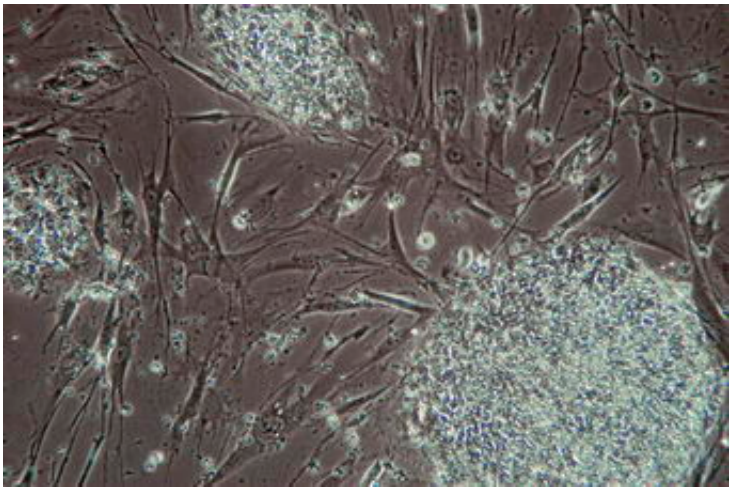

Image: Microscopic view of a colony of original human embryonic stem cell lines. Credit: Jeff Miller.

Scientists working at the WiCell Research Institute, a private laboratory affiliated with the University of Wisconsin-Madison, have developed a precisely defined stem cell culture system free of animal cells and used it to derived two new human embryonic stem cell.

The new work, which is reported in the journal *Nature Biotechnology* (Jan. 1, 2006), helps move stem cells a small step closer to clinical reality by completely ridding the culture medium in which they are grown of animal products that could harbor viruses or other deleterious agents.

Successfully growing living cells outside the body generally requires providing the cells in a lab dish with the right mix of nutrients, hormones, growth factors and blood serum. But those methods have often depended on animal cells - such as those obtained from mouse embryos in the case of embryonic stem cells - and other animal products to keep the cells alive and thriving in culture. Some scientists worry that animal viruses and other problematic agents might be taken up in the human cells and infect human patients, should those cells be used for therapy.

"All of the concerns about contaminating proteins in existing stem cell lines can essentially be removed using this medium," says the Nature Biotechnology paper's lead author, Tenneille Ludwig, a UW-Madison research scientist working at WiCell who led the effort to develop the new culture media. "This work helps us clear some of the major hurdles for using these cells therapeutically."

"We've been optimizing (culture) media on the existing stem cell lines since 1998, but it has only been recently that there have been dramatic improvements," says James Thomson, the senior author of the new study and a UW-Madison professor of anatomy who seven years ago was the first to successfully grow human embryonic stem cells in the lab. "This is the first time it has been possible for us to derive new cell lines in completely defined conditions in medium that completely lacks animal products."

Other groups, Thomson notes, have cultured established human embryonic stem cells in animal-product free media, but those efforts included the use of poorly defined or proprietary products, and no one has previously demonstrated derivation of new cell lines in defined conditions.

The two new Wisconsin stem cell lines have survived for more than

seven months in the new culture medium. Thomson says one of the new lines had an abnormal chromosome at four months, while the second line initially was normal but developed an abnormality by seven months.

In addition to testing the new stem cell culture medium on new lines, Thomson's group successfully cultured four existing stem cell lines in the new culture mix for extended periods, and their chromosomes remained normal.

"It is unclear how much these changes are related to the medium at this point, as we have occasionally observed similar changes in previous culture conditions," he says. "However, these changes do indicate that that further work is needed to understand chromosome stability of stem cells during long-term culture."

In early 2005, WiCell scientists reported that they were able to culture stem cells in the absence of mouse feeder cells, the most prominent animal product in stem cell culture systems. The new work effectively removes remaining animal products such as bovine serum and replaces them with products of human origin in a recipe that is completely defined.

But while the new Wisconsin work represents an important advance for stem cell technology, the ability to now grow embryonic stem cells in cultures free of animal products could accentuate the debate over federal funding for research on additional stem cell lines. The Senate is expected to take up the issue early in 2006 when it debates legislation that would expand funding to lines derived after August 2001, when President Bush imposed a compromise enabling federal funds to be used for the first time to study a limited number of established stem cell lines.

"Derivation and culture in serum-free, animal product-free, feeder-independent conditions mean that new human (embryonic stem) cell

lines could be qualitatively different from the original lines, and makes current public policy in the United States increasingly unsound," the authors of the new Nature Biotechnology report conclude.

While the 2001 compromise effectively opened up the field of embryonic stem cell research in the United States, researchers have been calling for expanding the number of lines approved for federal funding because existing lines have been shown to accumulate genetic mutations that, while not a serious impediment to scientific research, make the lines questionable for therapeutic use. What's more, some scientists say additional lines are needed to ensure broad diversity in order to better match patients with cells and gain a more comprehensive understanding of disease as it affects people with different genetic backgrounds.

The original five stem cell lines derived by Thomson in 1998, like many subsequently derived lines, depend on tissue culture technology that was established in the 1950s. In addition to mouse feeder cells obtained from mouse embryos, the original embryonic stem cell cultures depended on bovine serum products.

The two new Wisconsin stem cell lines were derived from five blastocysts, embryos less than a week old and which were donated for research with the informed, written consent of patients who were no longer undergoing treatment for infertility. The experiments were reviewed and approved by the UW-Madison's institutional review board, a panel that oversees work with human subjects.

Source: University of Wisconsin-Madison