Bush embryonic stem cell lines different from newly derived cell lines

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Established human embryonic cell lines, including those approved for federal research funding under former President George W. Bush, are different than newly derived human embryonic stem cell lines, according to a study by UCLA stem cell researchers.

The finding, by scientists with the Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research at UCLA, points to the importance of continuing to derive new stem cell lines so researchers can better understand pluripotency, the ability of these cells to make every cell in the human body, said study senior author Amander Clark, an assistant professor of molecular, cell and developmental biology in Life Sciences.

"It is critical to find out the characteristics that result in the highest quality pluripotent stem cell lines that we can make," Clark said. "It is possible that we have not set the bar high enough yet for embryonic stem cells or induced pluripotent stem cells. We now know that established lines are different from newly derived lines and now we have to find out how important that is."

The study appears Nov. 30, 2011 in the early online edition of the peer-reviewed journal Human Molecular Genetics.

The study looked at the first six human embryonic stem cell lines developed by Clark's research team at UCLA from 2009 to 2011, which have since been accepted by the National Institute of Health's embryonic

stem cell registry, founded by executive order in March 2009. Acceptance into the registry allows the UCLA lines to be used in federally funded research projects.

In her study, Clark decided to examine X chromosome inactivation and the mechanisms by which female stem cells turn off one X chromosome during development because it is a large physical marker that is easy to visualize in individual cells. Clark wanted to compare this specific molecular signature in established embryonic stem cell lines versus what occurs during the derivation of new embryonic stem cell lines from human blastocysts.

The established lines examined in the study were from a group of stem cell lines derived prior to 2001. The field has known for many years that the majority of established lines, Clark said, had already undergone X chromosome inactivation, and her work confirmed this finding. However, with the progression of time, Clark found that the molecular signature no longer reflected the normal process of X chromosome inactivation.

The X chromosome normally is inactivated by non-coding RNA and a special form of chromatin in female cells. In abnormal states, such as those found in the older, established human embryonic stem cells, the X chromosome is inactive, but this process is not regulated by the non-coding RNA and the chromatin is different.

"The classic signature is gone, so something else is regulating X chromosome inactivation in the established cell lines," Clark said. "It will be important not only to find out what that is, but also to discover what else is changing in the nucleus that we cannot see regardless of whether the cell line is male or female."

The new cell lines generated by Clark's research team were derived from

human embryos that were donated to the Broad Stem Cell Research Center by couples who had previously undergone in vitro fertilization to overcome infertility. The couples no longer planned to store or use their frozen embryos for reproductive purposes and had declined to donate the embryos to others for reproductive use.

The human embryos were transferred from the fertility clinic to the derivation lab at UCLA in frozen vials. They were then thawed by Clark's research team, and at six to seven days of development the embryos, or blastocysts, contained a cluster of cells called the inner cell mass. The inner cell mass is the source of new embryonic stem cell lines.

Clark's lab examined the human embryonic stem cell lines three to four weeks after growth from the inner cell mass and found that both X chromosomes were still active in many cells, making them more like the cells from the original inner cell mass.

Slowly, with time in culture and cryo-preservation – how the lines are ultimately stored - one X chromosome is inactivated and the cell lines become identical to the older, established lines, including abnormal X chromosome inactivation, Clark said.

The question, Clark said, is whether the first cells to grow out from the inner cell mass are of a higher quality, and therefore the ones researchers should be aspiring to use for research and potentially therapeutically.

"It may prove to be important to stabilize these cells at that very young state, one that's closest in identity to the inner cell mass," Clark said. "And then we can ask whether these cells give the best quality when differentiated into clinical cell types."

Keeping both X chromosomes active will also be important in modeling diseases such as Rett syndrome.

Going forward, Clark will study human embryonic stem cells in three states, lines in which the X chromosome is inactivated by normal means, lines in which the chromosome is inactivated abnormally and lines in which both X chromosomes remain active. Clark will seek to understand the differentiation potential of each of the three states.

"Our data highlights the importance of maintaining human embryonic stem cell derivation efforts. Gold standard human embryonic stem cell lines should be the benchmark for all human pluripotent stem cell research," the study states. "Developing new experimental approaches aimed at sustaining human pluripotent nuclei in an epigenetic state closer in identity to the day six or seven day human blastocyst is to work towards a more robust gold standard."

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