Unit 1: Paper Summary

Authors: Irina Klimanskaya, Young Chung, Sandy Becker, Shi-Jiang Lu, and Robert Lanza.

"Human embryonic stem cell lines derived from single blastomeres."

Nature 444 (November 2006): 481-485.

Abstract

The derivation of human embryonic stem (hES) cells currently requires the destruction of ex utero embryos. A previous study in mice indicates that it might be possible to generate embryonic stem (ES) cells using a single-cell biopsy similar to that used in preimplantation genetic diagnosis (PGD), which does not interfere with the embryo's developmental potential. By growing the single blastomere overnight, the resulting cells could be used for both genetic testing and stem cell derivation without affecting the clinical outcome of the procedure. Here we report a series of ten separate experiments demonstrating that hES cells can be derived from single blastomeres. In this proof-of-principle study, multiple biopsies were taken from each embryo using micromanipulation techniques and none of the biopsied embryos were allowed to develop in culture. Nineteen ES-cell-like outgrowths and two stable hES cell lines were obtained. The latter hES cell lines maintained undifferentiated proliferation for more than eight months, and showed normal karyotype and expression of markers of pluripotency, including Oct-4, SSEA-3, SSEA-4, TRA-1-60, TRA-1-81, nanog and alkaline phosphatase. These cells retained the potential to form derivatives of all three embryonic germ layers both in vitro and in teratomas. The ability to create new stem cell lines and therapies without destroying embryos would address the ethical concerns of many, and allow the generation of matched tissue for children and siblings born from transferred PGD embryos.

This article is a “proof of principle” paper describing a technique in which an embryonic stem cell line could be derived from a single cell of a human embryo. This technique is an adaptation of the procedure used during Preimplantation Genetic Diagnosis, where a single cell is removed from an embryo prior to implantation. The embryo can survive this procedure and be implanted in a woman if the embryo is shown to be genetically normal. The authors suggest that this could be a method of obtaining new stem cell lines without actually destroying the embryo. However it is important to note that in this study *multiple cells* were taken from each of the embryos in order to minimize the

number of embryos that were used. In this process the embryos were destroyed. The paper simply aims to demonstrate that the procedure is possible.

The researchers obtained 16 human embryos produced by in vitro fertilization for clinical purposes with informed consent of the donors. These 16 embryos were observed to be physically normal, where all the cells within the embryo were the same size and they appeared to be dividing normally. Unhealthy embryos were not used in this study. The embryos were grown to the 8-10 cell stage, where the cells are totipotent, and then multiple individual blastomeres (embryonic cells) were plucked away using a micropipette. In total the researchers obtained 91 cells in this manner. Each of these individual cells was cultured with mouse feeder cells and human embryonic stem cells from an already-established stem cell line. 53 blastomeres (cells) divided at least once in culture and 23 appeared to have embryonic-stem-cell-like growth within 2 days. The human embryonic stem cells that they added express a green fluorescent protein so that later the researchers can tell the difference between embryonic stem cells they added (green) and cells they made from the single blastomeres (normal color).

After several days the researches saw one of three things in the cultures. 1) The cells began to differentiate. 2) The cells looked like trophectoderm[i]. 3) The cells proliferated and appeared to be ES cells. Only two of the cell lines looked like ES cells, and these were analyzed further. 2 out of 91 seems like a small number, but it is actually similar to the percentage at which ES cells can be successfully derived from more established methods. The authors point out that the two cell lines were obtained from embryos that were rated in the healthiest group.

In order to confirm that these cells were in fact embryonic stem cells they performed antibody staining and found the lines expressed many markers of pluripotency: Oct4, Sea3, Sea4 , Tra-1-60, and Tra-1-80. It is common for stem cells to develop aneuploidy, which means cells have an abnormal number of chromosomes. The researchers determine that their derived stem cell lines had a normal number of chromosomes. They also determined that these cells were different from the ES cells they were cultured with because they did not contain Green Fluorescent Protein. Also the two new lines were determined to be female and contained two X chromosomes, while the ES cells they were cultured with were male (XY).

An important test of embryonic stem cells is that they can differentiate into cells of the three embryonic germ layers: the ectoderm, mesoderm and endoderm. When the cells were allowed to overgrow they formed clumps of cells called embryoid bodies. These were antibody stained and were shown to have markers for each of the three layers. They were also able to in vitro (in tissue culture dishes) differentiate the cells into specific cell types, like blood-vessel-like cells and skin cells. They also show that when

injected into the liver of an immunodeficient mouse, these cell form tumor like teratomas, which is another classic test of pluripotent stem cells.

The researchers conclude this study by examining future directions and possible problems with this method. First in order to use cells derived in this way they would need to find a replacement for the mouse feeder cells. Animal cells might have diseases and aren't considered safe in medical therapies. Secondly they indicate more study is needed to determine if there are any differences between single-cell-derived ES cells and ES cells derived from 5-14 day embryos. It is possible that the cells may not differentiate into specific cell types as easily as traditional ES cells. Also they address the concern that cells at the 8-10 cell stage are totipotent and could in theory become a whole embryo. They conclude though that a single cell without any signaling molecules would not be able to develop into a human being. They end by cautioning that 1) the safety of this method has not been established and 2) that it only be used if Preimplantation Genetic Diagnosis is already being performed.

---

[i] This makes sense because researchers were culturing totipotent stem cells—ones that can still form the trophoblast (which becomes the placenta and supporting tissues) as well as cells in the developing fetus. Trophectoderm will later differentiate into (become) these trophoblastic cells. Pluripotent stem cells extracted from the inside of 5-14 day blastocysts would not be able to form trophoblast cells or placenta because they have passed the totipotent morula stage.