

## Activity 2: A Source of Embryonic Stem Cells: Human Clones?

### Assignment: Reading Scientific Literature

When reading a paragraph in a textbook, you might take for granted that the information is presented clearly, free of ambiguities or conflicting data. You might be surprised to learn that several years of research and many papers by professional scientists, post-doctoral fellows, and students contributed to that one paragraph. Still more surprising is that just one paragraph represents the final consensus regarding data that came from collaborating or competing research groups. Some articles are written for scientists researching within the same field, while others are written for those with only a general scientific background or no formal scientific training at all. In this assignment, you will read review articles, news articles, and more technical research articles.

Research articles are written by scientists in a specific area of research for other scientists working in that area. These articles aim to report a unique contribution to the field and focus on the technical details of a particular experiment and the wider implications of those results. Manuscripts submitted for publication undergo peer review, and if accepted for publication, indicate that the research was conducted in a rigorous manner upheld by those in the field. Collectively, research articles document the incremental process of scientific discovery and present alternative points of view and are referred to as the "primary literature" of the field. After publication, research articles receive commentary by other researchers in the field and these are published alongside responses in subsequent issues of the journal. The scientific community can question the results or ethics of the study and ask authors to explain discrepancies. If the research study can not withstand this scrutiny or address the questions, the article may be retracted.

It is important to note, that as in this activity, it may appear that the research moved forward in a relatively linear fashion with few unexpected or negative results. However, as the news and commentaries demonstrate, this is usually not the case. Often scientists within a research field will be aware of the setbacks and disappointments of experimentation by learning of them through conference proceedings, abstracts, and conversations. Therefore, students outside the field of research must be thorough in reading not only the primary literature, but expand their scope to include interviews, scientific reviews, and news articles that reveal the conundrums and complexities of the science.

These latter texts are considered part of the "secondary literature" of the field, and are written to attract the interest of scientists and students who are not experts within a particular field. These articles usually provide a synopsis of the state of affairs within that field and give the non-expert an overview of recent advances and controversies. News articles, aimed at the general public, will contain interviews with the scientists, presenting the reader with a personality profile depicting a stereotypical scientist, or perhaps, attempt to humanize him or her. In general, these texts are often free of scientific jargon that can intimidate or bog down the novice. When conducting research in an unfamiliar field of biology, then, it is useful to start with news and review articles and then move on to research articles.

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In this assignment, you will learn how to approach a field of scientific research for the first time. In reading the literature assigned here, compare the genres and notice particularly the differences in titles, formats, and language that reflect the purposes of the different genres. The research article in this activity is among the seminal papers reporting that human blastocysts can be cloned and that embryonic stem cells (ESCs) from these clones are capable of adopting many cell fates upon stimulation *in vitro* and *in vivo*. The authors report that human cloning can provide a source of ESCs and point the way to the future of stem cell technologies used to treat diseases and to expand our current understanding of genetics and cell and developmental biology.

Because research like this is so ground breaking, a manuscript often undergoes expedited peer review and can result in early electronic publication. Many journals now have an *Express* version and articles will appear here first and then in the hard copy publication a few weeks later. A research article that will have larger implications for society as a whole will often be sent to science journalists five days before publication, so that they may get up to speed on the science and accurately report the findings when the article is released. As the last question in this assignment indicates, preliminary research may be announced to the general public before peer-review, resulting in a backlash of criticism. In this assignment, you will read both primary and secondary literature and learn about work before and after the publication of a series of research articles published by stem cell researchers using the controversial somatic cell nuclear transfer (SCNT) technique to establish human stem cell lines.

### Instructions

1. Review the assigned textbook sections (suggestions may be found in the Teaching Notes to Activity 2).
2. Read the assigned articles. Begin with the news and review articles and then use **Resource Seven: Worksheet for Reading Primary Literature** to assist in reading the primary research article.
3. Watch for the main points and experimental methods listed under the Key Ideas heading. Define the key terms and use the web sites listed in the **Student Vocabulary Aid for Activity 2** to get started on building your knowledge.
4. Answer the questions listed below. They are designed to help you decipher the meaning of these papers and understand the relationships between the figures, tables, and text. Some of the questions are challenging, and the answers may not be in the reading. Apply your skills and knowledge to construct the answer.
5. Your instructor may ask you to submit your answers for grading or ask you to discuss or present what you have learned to the rest of the class.

### Readings

#### Research Article:

Hwang, W. S. et al. (2004). "Evidence of a pluripotent human embryonic stem cell line derived from a cloned blastocyst." *Science* 303 (5664): 1669-1674. Originally published on February 12, 2004 in *Science Express*. Retracted in 2006.

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### News Articles:

Recer, P. (2004). "Korea's Successful Human Cloning to Obtain Stem Cells Sparks Calls for US Ban." *SympaticoMSN.Com*. February 12.

[http://mediresource.sympatico.ca/health\\_news\\_detail.asp?channel\\_id=28&menu\\_item\\_id=&news\\_id=3353](http://mediresource.sympatico.ca/health_news_detail.asp?channel_id=28&menu_item_id=&news_id=3353)

Faiola, A. (2004). "Dr. Clone: Creating Life or Saving It?" *Washington Post*. February 29:01. <http://www.washingtonpost.com/ac2/wp-dyn/A15746-2004Feb28?language=printer>

Cyranoski, D. (2004). "Korea's stem-cell stars dogged by suspicion of ethical breach." *Nature* 249(6987): 3. <http://www.nature.com/nature/journal/v429/n6987/full/429003a.html>. **OR**

Normile, D. (2004). "South Korean cloning team denies improprieties." *Science* 304(5673): 945.

Fairclough, G. (2005). "Women Offer Eggs to Doctor in South Korea." *Wall Street Journal*. November 25: 1

Kevles, B.H. (2006). "Barely a Drop of Fraud; Why It Shouldn't Taint Our View of Science." *The Washington Post*. January 8: 03.

Fifield, A. et al. (2006). "Seoul Panel Confirms Stem Cell Fraud." *Financial Times*. January 11: 7.

Normile, D. et al. (2006). "South Korean team's remaining human stem cell claim demolished." *Science* 311(5758): 156-157.

Wade, N. (2006). "It May Look Authentic; Here's How to Tell It Isn't." *The New York Times*. January 24: F1,6.

Templeton, S.-K. (2006). "Cloning Expert Quits Country in Row With Partner." Online. Times Newspapers Ltd.. January 15. <http://www.timesonline.co.uk/article/0,,2087-1986322,00.html> .

### Bioethics Articles:

Magnus, D. et al. (2005). "Issues in oocyte donation for stem cell research." *Science* 308(5729): 1747-1748. Originally published on May 19, 2005 in *Science Express*.

Hyun, I. (2006). "Fair payment or undue inducement?" *Nature* 442(7103): 629-30.

Jung, K.-W. et al. (2006). "RETRACTED: Oocyte and Somatic Cell Procurement for Stem Cell Research." *The American Journal of Bioethics* 6(1): W19.

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### A Selection of Recommended Background Reading:

- University of Michigan. (2006). "Stem Cells Explained and Explored." Online. University of Michigan. March 21. This site reviews ASC and ESC procurement and their applications with good animations.  
[http://www.umich.edu/news/stemcells/022706\\_TabA.html](http://www.umich.edu/news/stemcells/022706_TabA.html) or  
<http://www.lifesciences.umich.edu/research/featured/tutorial.html>.
- *Scientific American* and *The Financial Times*. (2005). The Future of Stem Cells: Special Supplement. June 20. This supplement has several articles which cover a wide range of subjects including therapy, law, and business. <http://news.ft.com/reports/stemcells2005>.
- Shaw, J. (2004). "Stem-cell Science: When Medicine Meets Moral Philosophy." *Harvard Magazine* July-August 106(6): 36-45. A comprehensive review of the potential for stem cell therapy. <http://www.harvardmagazine.com/on-line/070483.html>
- Lanza R., Rosenthal N. (2004) "The Stem Cell Challenge." *Scientific American.com*. 290(6): 92-100. A comprehensive review of the field.  
[http://www.sciam.com/print\\_version.cfm?articleID=000DFA43-04B1-10AA-84B183414B7F0000](http://www.sciam.com/print_version.cfm?articleID=000DFA43-04B1-10AA-84B183414B7F0000)
- Rohm, W. (2004). "Seven Days of Creation." *Wired*. 12.01 January. This feature article is part of a cover story on cloning and reviews the work of Chung at Advanced Cell Technology. <http://www.wired.com/wired/archive/12.01/clones.pr.html>
- Hall, S. (2000). "The Recycled Generation." *New York Times Magazine*, 149 (51823): 30 (10 pages). A feature article about the work of Advanced Cell Technology, scientific researcher Jose Cibelli, and its former CEO Michael West.  
<http://www.nytimes.com/library/magazine/home/20000130mag-hall6.html>

### Key Ideas

**Main points:** Researchers in South Korea report that they can obtain embryonic stem cells (ESCs) from a cloned human blastocyst. They claim to have achieved this landmark discovery using somatic nuclear transfer (SCNT) technology and expertise in the veterinary sciences. The human ESCs were shown to differentiate into all major cell types *in vitro* and *in vivo* in an experimental mouse system.

**Experimental methods:** SCNT, karyotyping, immunocytochemistry, RT-PCR, DNA fingerprinting, and genomic imprinting analyses.

### Scientific Terms:

To help you define the terms below, use the supplemental **Student Vocabulary Aid for Activity 2** for this activity, which has links for each

fertilization and embryogenesis  
oogenesis, zona pellicuda, and cumulus cells  
ovarian stimulation/HCG

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calcium activation wave/calcium ionophore  
SCNT  
parthenogenesis  
teratoma/embryoid bodies  
Karyotyping and Mitomycin C  
LIF and gp130  
SCID Mouse  
immunocytochemistry (direct and indirect)  
pronase  
Oct4  
genomic imprinting  
gel electrophoresis  
RT-PCR, STR, and DNA polymorphism

### Questions

1. What is the precedent for the work described in this research article? Specifically, what work does it build it on? Is this paper an extension of previous work or does it aim to present an alternative point of view?
2. Summarize the study, and highlight the salient scientific methods and results. Pay close attention to how the researchers learn more about stem cells and cloning. In other words, which experimental methods point to which set of results?
3. How do the results in this research article affect the wider field of stem cell research? How have these results affect national and international regulations?
4. Based on the results presented in this research article, what scientific questions remain? Can you propose experiments for the future? Do you feel that these future experiments are ethical and in line with university Institutional Review Board missions to protect the rights of human subjects who participate in research? Explain your reasoning and provide suggestions that ensure informed consent and reduce conflict of interests and exploitation. Be sure to consider whose rights might be violated and whose health might be at risk (In the U.S. Code of Federal Regulations, Title 45, part 46 deals specifically with this issue and the informed consent protocol for this study is available in the supplemental materials provided with the online version of this article).
5. Recer reports that the South Korean cloned blastocysts were only established when the donor and the recipient were the same person (autologous SCNT). Given what you know about sexual reproduction, hypothesize as to why heterologous SCNT was unsuccessful. Why would this information be eliminated from the research article?
6. In Table 1 of the Hwang article, four sets of experiments are displayed. Reflect on the scientific method, and explain how Hwang and Moon's group followed this methodology (be detailed in your explanation describing the controls, variables, sample size, etc. in

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each set of experiments). Look closely at the table and explain why different results are obtained in the 2<sup>nd</sup> set of experiments (line 1 and line 3).

7. In Figure 1 of the research article, the human SCNT embryonic cells (SCNT-hES-1) cells are stained for the presence of the protein nestin. Why did the researchers choose to detect this protein specifically and what did you think they added to the SCNT-hES-1 cells to prompt them to transcribe this gene? Given the pattern of staining, what role do you think nestin plays in the cell?
8. In Figure 2 of the research article, the negative control is defined as “not treated with first antibodies.” How does the elimination of this step of indirect immunocytochemistry serve as a negative control? Draw two pictures that depict the process with and without the “first antibody” and the resulting outcomes. What serves as the positive control for this experiment?
9. In Figure 3 of the research article, the scientists demonstrate that the transplanted SCNT-hES-1 cells are not only accepted by the experimental mouse system but are capable of differentiating into different cell types.
  - a) Why doesn't the mouse reject the foreign human ES cells?
  - b) Briefly describe two different conceptual models for the differentiation of these human ES cells into different cell types in the mouse.
10. In Figure 4, A-C of the research article, DNA fingerprinting techniques are used to establish that the resulting embryo was a product of SCNT and not parthenogenesis. How would these results look if the embryo had resulted from parthenogenesis (for this question you must consider the events of oogenesis)?
11. Are the results in Figure 4, D of the research article more convincing in terms of establishing that the cloned embryos were a result of SCNT? What are the positive and negative controls for this experiment and what do the bands in the figure represent?
12. In the assay represented in Figure 4, D of the research article, one gene that codes for the enzyme GAPDH is called a “housekeeping” gene in the methods. What does this phrase mean?
13. In 2005, the Hwang and Moon research team collaborated with a number of other stem cell researchers and published another ground breaking research article. In this article, it was reported that eleven patient-specific embryonic stem cell lines were isolated and characterized from cloned human blastocysts. What methods did they use to surpass their reported achievements in 2004? What implications would results like this have for the future of clinical medicine?
14. As early as 2004, a report regarding the unethical procurement of eggs from donors appeared in the journal *Nature*, and in late 2005, the media was informed by one of the members of the research team that results presented in the research papers were falsified

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and exaggerated the success rate of blastocyst production via SCNT. Gerry Schatten, an American stem cell researcher who collaborated with the Hwang team, immediately broke off ties with the team, but was later reprimanded for misconduct for his negligence and unethical behavior in this study. Though the scientific papers and the bioethics paper were retracted in early 2006, how might these papers be instructive to the stem cell research field?

15. Coincident with the 2005 Hwang announcement of more human cloned stem cell lines, researchers at the Newcastle University in the UK announced that they had also cloned a human blastocyst using embryonic stem cells as donors in nuclear transfer. In January 2006, however, one of the stem cell researchers Miodrag Stojkovic broke off his collaboration with Alison Murdoch citing unethical scientific practices. How might using embryonic stem cells as donors increase the efficiency of obtaining viable human cloned blastocysts? How did the announcement of this work break protocol with the norms in scientific communication? Both Stojkovic and Schatten disengaged with their collaborators upon hearing of suspicious scientific practices, but how might international regulation shape the nature of such future collaborations?