Nuclear Cloning, Embryonic Stem Cells and Cell Therapy: Promise, Problems, Reality

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The full term development of sheep, cows, goats, pigs, and mice has been achieved through the transfer of somatic cell nuclei into enucleated oocytes. Despite these successes, mammalian cloning remains an inefficient process, with a preponderance of reconstructed embryos failing at early to midgestation stages of development. The small fraction of conceptuses that survive to term are characterized by a high mortality rate and frequently display grossly increased placental and birth weights. It is likely that inappropriate expression of crucial developmental genes may contribute to lethality of cloned embryos.

One of the key issues raised by nuclear cloning is the question of genomic reprogramming, i.e. the mechanism of resetting the epigenetic modifications that are characteristics of the adult donor nucleus to ones that are appropriate for an embryonic cell. Also, a major unresolved question that was raised half a century ago in the seminal nuclear transfer experiments with amphibians is whether nuclei of terminally differentiated cells can be reprogrammed to direct development of a new organism. To address these questions we have used ES cells, terminally differentiated lymphoid cells (Hochedlinger and Jaenisch 2002), neurons (Eggan et al. 2004) and cancer cells (Hochedlinger et al. 2004) as donors for nuclear transplantation. Our results show that the efficiency of generating cloned mice from an embryonic donor cell nucleus is significantly higher than cloning from a terminally differentiated donor cell nucleus. This is consistent with the notion that the differentiation state of the donor nucleus profoundly affects the efficiency of epigenetic reprogramming after nuclear transfer into the egg. Indeed, expression analyses indicated that the failure to properly activate key embryonic genes that are constitutively expressed in the embryonic but not in the adult donor cell represents one of the most important causes for early clone demise. Likely this is due to the genome of an adult cell being less amenable to the reprogramming activity of the oocyte than that of an embryonic cell (Jaenisch et al. 2004). It is crucial to understand the molecular circuitry in a stem cell and that in a differentiated somatic cell. I will discuss our progress in understanding the key regulatory mechanisms that are crucial for self-renewal of stem cells (Boyer et al. 2005) and our approach in using such information to understand and possibly improve the low efficiency of genomic reprogramming after nuclear transplantation.

An emerging consensus is that somatic cell nuclear transfer (SCNT) for the purpose of creating a child (also called "reproductive cloning") is not acceptable for both moral and scientific reasons. In contrast, SCNT with the goal of generating an embryonic stem cell line ("therapeutic cloning") remains a controversial issue (Jaenisch 2004). Although therapeutic cloning holds the promise of yielding new ways of treating a number of degenerative diseases, it is not acceptable to many because the derivation of an embryonic stem cell line from the cloned embryo (an essential step in this process) necessarily involves the loss of an embryo and hence the destruction of potential human life. I will describe a proof of principle experiment of the "Altered Nuclear Transfer" (ANT) approach (Meissner and Jaenisch 2006), a modification of SCNT that has been suggested by Hurlbut (2005). The purpose of ANT is

to avoid the generation of embryos that have any potential for fetal development but still can be the source of "customized" ES cells for cell therapy. The intention of this experiment has been to provide a basis for a more rational discussion of these complex issues.

References

Boyer, L. A., Lee, T. I., Cole, M. F., Johnstone, S. E., Levine, S. S., Zucker, J. P., Guenther, M. G., Kumar, R. M., Murray, H. L., Jenner, R. G., Gifford, D. K., Melton, D. A., Jaenisch, R., and Young, R. A.: Core transcriptional regulatory circuitry in human embryonic stem cells. Cell *122*, 947–956 (2005)

Eggan, K., Baldwin, K., Tackett, M., Osborne, J., Gogos, J., Chess, A., Axel, R., and Jaenisch, R.: Mice cloned from olfactory sensory neurons. Nature *428*, 44–49 (2004)

Hochedlinger, K., Blelloch, R., Brennan, C., Yamada, Y., Kim, M., Chin, L., and Jaenisch, R.: Reprogramming of a melanoma genome by nuclear transplantation. Genes Dev. *18*, 1875–1885 (2004)

Hochedlinger, K., and Jaenisch, R.: Monoclonal mice generated by nuclear transfer from mature B and T donor cells. Nature *415*, 1035–1038 (2002)

Hurlbut, W. B.: Altered nuclear transfer as a morally acceptable means for the procurement of human embryonic stem cells. Perspect. Biol. Med. *48*, 211–228 (2005)

Jaenisch, R.: Human cloning – the science and ethics of nuclear transplantation. New Engl. J. Med. *351*, 2787–2791 (2004)

Jaenisch, R., Hochedlinger, K., Blelloch, R., Yamada, Y., Baldwin, K., and Eggan, K.: Nuclear Cloning, Epigenetic Reprogramming, and Cellular Differentiation. Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor Press Vol. *LXIX*, 19–28 (2004)

Meissner, A., and Jaenisch, R.: Generation of nuclear transfer-derived pluripotent ES cells from cloned Cdx2-deficient blastocysts. Nature *439*, 212–215 (2006)