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RESEARCH HIGHLIGHT

ES cells derived from cloned embryos in monkey – a jump toward human therapeutic cloning

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Therapeutic cloning refers to the derivation of embryonic stem cells (ntESC) from embryos derived from somatic cell nuclear transfer (SCNT) also known as cloning. Cloning involves transplanting a differentiated cell into an oocyte that has had its nucleus (DNA) removed. The reconstructed oocyte can be activated to divide and develop into an embryo. The process that allows this to happen is termed nuclear reprogramming, and is defined as the mechanism through which a differentiated cell de-differentiates or returns to a totipotent state (capable of giving rise to any cell type, including extra-embryonic) and directs embryonic development [1]. Cells from blastocyst stage cloned embryos can be used to generate ntESC lines. Such cell lines can differentiate into any adult cell type, and have tremendous potential for patient-specific disease therapy [2]. This is based on the principle that cells from a patient can be used in the cloning procedure. Subsequently, ntESC lines can be derived from cloned embryos and undergo targeted differentiation. Upon differentiation into the desired cell type, the ntESC can be used to treat the patient without the threat of immune rejection. This is in contrast to ESC lines that have been generated from surplus *in vitro* fertilized (IVF) embryos, which upon transfer to a patient would be perceived as non-self and rejected. Thus, the treatment of a multitude of serious diseases could be made possible by therapeutic cloning. Toward this end, progress has been made in mice and cattle as ntESC lines have been derived from cloned blastocysts [1]. However reports of human ntESC lines have been discredited and until now little success has been made in the arena of primate cloning. Thus, the generation of monkey ntESC lines by Bryne *et al*. [3] demonstrates a significant jump toward determining the feasibility of human therapeutic cloning.

Cloning is difficult in non-human primates, where reconstructed oocytes show low efficiency of development to blastocyst stage [1]. The study by Bryne *et al*. shows dramatic improvement in blastocyst rates (up to 16%) through the utilization of an Oosight™ spindle imaging system that allowed for the visualization and complete removal of the oocyte DNA without detrimental effects to the embryo. The authors discuss how the previous method of using Hoechst 333342 dye and UV light for spindle visualization could be damaging and affect rhesus monkey embryonic development. This increased efficiency of embryonic development led to the derivation of 2 ntESC lines from 20 blastocysts. An ESC line derivation efficiency of 10% is within the range that is seen in mice and cattle in previous studies. The authors confirmed that the ntESC lines were indeed derived from the 9-year old male Rhesus monkey by analyzing polymorphic markers (microsatellite and single nucleotide polymorphism SNP). The ntESC lines demonstrated typical ESC morphology and were maintained in an undifferentiated state in culture. Additionally, both ntESC lines expressed the pluripotency (capable of giving rise to all cell types in the body) markers *OCT4* (*POU5F1*), *SSEA-4*, *TRA1-60* and *-80*, *NANOG*, *SOX2*, *LEFTY-A*, *TDGF* and *TERT*. Upon targeted *in vitro* differentiation using conditions favorable to cardiomyocyte development, the ntESCs formed contracting aggregates and expressed cardiac muscle markers. They were also directed toward neural differentiation and displayed correct neuronal cell morphology and expression of neuronal markers. Lastly for the *in vivo* measurement of pluripotency, ntESCs were injected into immune-deficient mice and the subsequent formation of tumors (teratomas) that contained tissues from the three germ layers was observed.

The authors went a step further in establishing the normality/pluripotency of the ntESC lines by examining

their transcriptional profiles via microarray technology. Previously, the transcription profiles of mouse ntESC lines were found to be indistinguishable from ESC lines derived from fertilized embryos [4, 5]. Byrne *et al*. demonstrate analogous findings with greater similarity in gene expression patterns when individual ntESC line replicates were compared with control ESC lines from IVF embryos than when the control ESC line replicates were compared with each other. Additionally, they showed a similar degree of departure in gene expression as the control ESC lines when compared with the profiles of the fibroblast donor cell line. Overall, this paper clearly describes the derivation and characterization of ESC lines from cloned monkey embryos and represents a significant advance in primate cloning. Additionally, it provides the necessary proof-of-concept and affords hope for the eventual establishment of human therapeutic cloning based treatment.

Even though the promise of human therapeutic cloning is great, a major hurdle is the requirement of human oocytes to reprogram the donor cell and the number that would be required due to the current low efficiency. Byrne *et al*. generated 2 ntESC lines from 304 oocytes collected from 14 rhesus monkeys, a 0.7% ntESC derivation efficiency based on the number of oocytes. Human oocytes are difficult to obtain and it is understandable that their collection raises ethical concerns about the potential risk to women donors and their compensation. However, human oocyte donation to help infertile couples has become a common practice worldwide because of the reasonable fertilization and pregnancy rates from the donated oocytes. Clearly, more research is needed to improve the efficiency of SCNT and ntESC technologies so that only a limited number of donated oocytes are needed to generate a patient-specific ntESC line. In the mouse model, it is now possible to generate a ntESC line from only 8-10 oocytes following SCNT. For human therapeutic cloning research, one approach to overcome the human donated oocyte limitation is to use oocytes from other species such as rabbits or cattle. Also, work in mice has shown that oocytes can be created by ESC *in vitro* differentiation [6]. Very recently, reports were published that showed direct reprogramming of mouse somatic cell lines into pluripotent ESC-like cell lines after retroviral transfection of four reprogramming factors [7-10]. Although this new capability would alleviate the need for oocytes and the nuclear transfer technology, the low efficiency and use of retroviruses could cause problems in a therapeutic setting. Thus, at this time, therapeutic cloning remains a promising technique for the treatment of many diseases and the study by Byrne *et al*. is the first to demonstrate its feasibility in primates.

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