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CELL TRANSFECTION & CELL FUSION PRODUCTS

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Electroporation facilitates derivation of Human Embryonic Stem Cells by Somatic Cell Nuclear Transfer

ABSTRACT

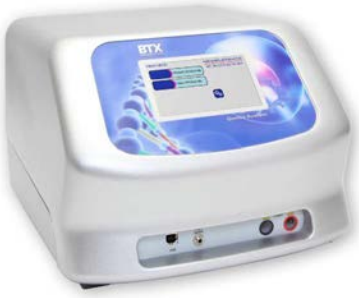
Reprogramming somatic cells into pluripotent embryonic stem cells (ESCs) by somatic cell nuclear transfer (SCNT) has been envisioned as an approach for generating patient-matched nuclear transfer (NT)- ESCs for studies of disease mechanisms and for developing specific therapies. Past attempts to produce human NT-ESCs have failed secondary to early embryonic arrest of SCNT embryos. The authors have identified premature exit from meiosis in human oocytes and suboptimal activation as key factors that are responsible for these outcomes. Optimized SCNT approaches designed to circumvent these limitations allowed derivation of human NT-ESCs. When applied to premium quality human oocytes, NT- ESC lines were derived from as few as two oocytes. NT-ESCs displayed normal diploid karyotypes and inherited their nuclear genome exclusively from parental somatic cells. Gene expression and differentiation profiles in human NT-ESCs were similar to embryo-derived ESCs, suggesting efficient reprogramming of somatic cells to a pluripotent state.

METHODS

Human SCNT Procedure- Cytoplast Activation

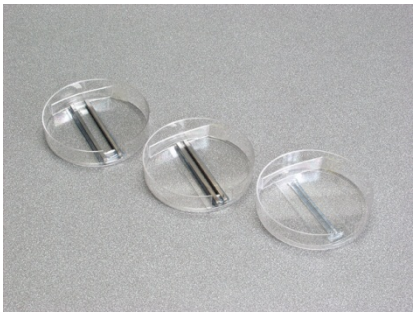
Enucleated oocytes and fibroblast fusion constructs were subjected to artificial activation consisting of electroporation pulses (two 50 ms DC pulses of 2.7 kV cm□1) (Electro Square Porator T-820, BTX) in 0.25 M d-sorbitol buffer containing 0.1 mM calcium acetate, 0.5 mM magnesium acetate, 0.5 mM HEPES, and 1 mg ml□1 fatty-acid-free BSA.

The equipment used in this study was a discontinued model of BTX. The same application can be performed with the new BTX Gemini X2 electroporation system.



Gemini X2 Generator
Cat. #452006

Microslide
Cat. # 450103, 450104



RESULTS

SCNT Protocol Optimization in a Nonhuman Primate Model

The authors demonstrated previously the human MII oocyte sensitivity to premature activation induced by removal and reintroduction of meiotic spindles (Tachibana et al., 2013) and to the use of electrofusion in the context of cytoplast activation (Tachibana et al., 2009). Consequently, their present investigation began with optimizing the use of envelope from inactivated hemagglutinating virus of Japan (HVJ-E) to fuse nuclear donor cells with enucleated MII oocytes while maintaining cytoplasts in meiosis (Tachibana et al., 2009). Because of limited oocyte availability, they first tested both the feasibility and efficacy of HVJ-E-based cell fusion on the development of rhesus macaque SCNT embryos. The fusion rate of adult fibroblasts with cytoplasts was 100% after HVJ-E treatment; however, and unexpectedly, the SCNT embryos generated by HVJ-E fusion failed to progress beyond the compact morula (CM) stage following standard ionomycin/DMAP (I/DMAP) activation. They previously demonstrated that monkey SCNT embryos produced by electrofusion developed into blastocysts (Byrne et al., 2007; Sparman et al., 2009). **Therefore, the exposure of the cytoplast to an electropulse (electroporation) could be beneficial for SCNT reprogramming, perhaps as a supplemental activation stimulus.** To test this possibility, they exposed HVJ-E-fused SCNT embryos to electroporation before the standard I/DMAP activation treatment. Ten percent of SCNT embryos were capable of reaching the blastocyst stage (Figure 1). Interestingly, this SCNT blastocyst formation rate was unaffected even when exposure to ionomycin was omitted and SCNT embryos were activated with electroporation followed by DMAP treatment (Figure 1). **Together, these results indicate that, although an electroporation stimulus is not required for cell fusion, it is supportive of proper cytoplast activation following SCNT.**

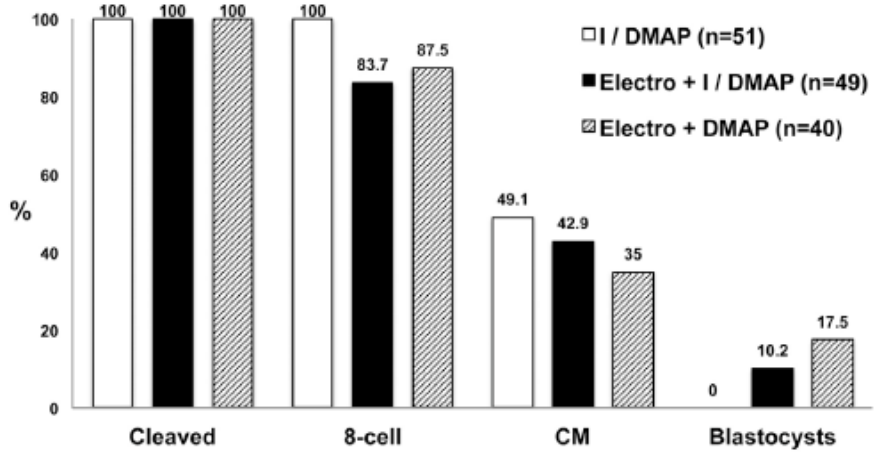


Figure 1. Development of Monkey SCNT Embryos Reconstructed with Optimized Protocols

Although HVJ-E fusion was efficient, SCNT constructs required activation by electroporation for blastocyst formation. Elimination of ionomycin from the activation treatment further improved blastocyst development. I, ionomycin; DMAP, 6-DMAP; CM, compact morula.

RESULTS

The authors demonstrate here for the first time the successful reprogramming of human somatic cells into ESCs following SCNT. By systematic analysis of SCNT procedures, in some cases informed by studies in the rhesus monkey, the authors have identified several steps, including spindle removal, donor cell fusion, and **cytoplast activation (provided by electroporation stimulus)**, that are critical for cellular reprogramming and SCNT blastocyst development.

Mitalipov et al., Human Embryonic Stem Cells Derived by Somatic Cell Nuclear Transfer. 2013, *Cell*