Toward the Generation of Haploid Human Oocytes by Somatic Cell Nuclear Transfer (SCNT)

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In vitro gametogenesis, or experimental production of gametes from somatic cells, could provide the opportunity for infertile families to have genetically related offspring. We present here an alternative method of producing haploid gametes by direct reprogramming using somatic cell nuclear transfer (SCNT).

Haploidization of human somatic cells was induced by premature cell division resulting after transplantation of fibroblasts at the G0/G1 stage of the cell cycle (2n2c) into the metaphase cytoplasm of enucleated mature oocytes. Metaphase oocyte cytoplasm induced rapid nuclear envelope breakdown, premature chromosome condensation and formation of bipolar spindles from the somatic cell chromatin in 81% of SCNT oocytes.

Artificial activation of such SCNT oocytes resulted in cell division and chromosome segregation into pseudo polar bodies and zygotic pronuclei at 70% efficiency. Next-generation sequencing of individual chromosomes in polar bodies and zygotes revealed that the numbers of chromosomes was reduced close to half (N=19) compared to the normal diploid (N=46) number in starting fibroblasts. Comprehensive sequencing of homologous pairs revealed that on average, half (N=11) of total 23 homologous pairs were properly segregated into a polar body and zygote while remaining chromosome pairs remained together resulting in aneuploidy. No evidence of recombination between somatic cell homologs was detected.

These results indicate that experimentally induced premature cell division after SCNT allows ploidy reduction albeit via random chromosome segregation suggesting that additional optimizations are required to aid in homolog pairing and segregation to achieve proper haploidy.