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Gene Expression in Somatic Derived Trophoblast Stem Cell (ntTS cell)

Satoshi Tanaka, Mayumi Oda and Kunio Shiota
Laboratory of Cellular Biochemistry, Animal Resource Sciences/Veterinary Medical Sciences, The University of Tokyo, Tokyo, Japan

Successes in the production of fully developed cloned animals by somatic cell nuclear transfer indicate that the nuclei of at least some somatic cells retain the potential to support embryogenesis when transferred into enucleated oocytes. In mammalian embryogenesis, trophoblast cells arise to form trophectoderm as the first functionally specified cells at the blastocyst stage. The trophoblast cells give rise to the greater part of the placenta. This in turn means that the first cell fate decision, which the donor somatic nuclei face in the development of the reconstituted embryos, occurs in the process of trophoblast cell differentiation, and that the epigenetic reprogramming of the transferred nuclei has to be accomplished by the time of blastocyst formation to allow successful development of the trophoblast cell lineage. Failure to reprogram the epigenetic trait on the genome of donor nucleus would cause impaired blastocyst formation or trophoblast malfunction. From this point of view, it is interesting to note that placental abnormalities have been observed in the production of cloned calves and mice. These results, obtained from two mammalian species whose placentation processes are quite different from each other, imply that there may be a common mechanism underlying the impact of somatic cell cloning on trophoblast development. We have previously reported that all of the trophoblastic layers in the term placenta of cloned mouse concepti show morphological abnormalities (1). We have also shown that there are aberrantly methylated regions in the genomic DNA of the placentas of the term cloned mice and identified *Sall3* locus as one of such regions (2, 3).

To analyze abnormalities in trophoblast cells of cloned mouse embryos in earlier developmental stage, we isolated trophoblast stem (TS) cells from cloned mouse embryos. Cumulus cell nuclei of C57BL/6 x DBA/2 F_1 hybrid (BDF1) mice were transferred into enucleated BDF1 oocytes, and 418 successfully activated, reconstituted oocytes were cultured *in vitro* for 4 days. Of them, 113 formed morphologically normal blastocyst and were individually used for TS cell derivation. Formation of primary TS cell colonies was, then, found in 63 wells after dissociation of 96 blastocyst outgrowths. This was comparable to the result obtained with naturally produced BDF1 blastocysts (22/24 attached, 14/22 formed primary TS cell colonies), suggesting that cloned embryos that developed to blastocyst stage have equivalent potential of producing TS cells. Considering that more than 90% of transferred NT embryos die in utero, this result also suggests that a majority of these primary TS cell colonies are derived from embryos that would not have developed to term if they were transferred to recipient females. Five individual TS cell lines (ntTS celllines) were established from these blastocyst-explants and used for further analyses. Northern hybridization analysis of a number of trophoblast-marker genes revealed that all ntTS cells indeed express stem cell markers but at aberrant level in some lines. Expression of differentiation-marker genes, such as Mash2 and Gcm1, was also disturbed both in level and timing of expression, suggesting that the developmental potential of ntTS cells are compromised. Abnormal expression of some placental growth-relatedimprinted genes was also found. We then compared global gene expression profiles of three ntTS and two control TS cell lines by DNA microarray analysis. Of approximately 12,000 genes analyzed, 2 and 3 were judged as decreased and increased, respectively, in all three ntTS cells lines, making those genes strong candidates for responsible genes of placental abnormality in cloned mouse concepti. The ntTS cells established in this study are powerful tool to analyze early abnormality in cloned embryos.

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