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Establishment of Bovine Trophoblast Stem-Like Cells from In Vitro-Produced Blastocyst-Stage Embryos Using Two Inhibitors

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The trophoblast (TR) is the first to differentiate during mammalian embryogenesis and play a pivotal role in the development of the placenta. We used a dual inhibitor system (PD0325901 and CHIR99021) with mixed feeders to successfully obtain bovine trophoblast stem-like (bTS) cells, which were similar in phenotype to mouse trophoblast stem cells (TSCs). The bTS cells that were generated using this system continually proliferated, displayed a normal diploid karyotype, and had no signs of altered morphology or differentiation even after 150 passages. These cells exhibited alkaline phosphatase (AP) activity and expressed pluripotency markers, such as *OCT4*, *NANOG*, *SOX2*, *SSEA-1*, *SSEA-4*, *TRA-1-60*, and *TRA-1-81*, and TR lineage markers such as *CDX2*, as determined by both immunofluorescence and reverse transcription-polymerase chain reaction (RT-PCR). Additionally, these cells generated dome-like structures, formed teratomas when injected into NOD-SCID mice, and differentiated into placenta TR cells in vitro. The microarray analysis of bTS cells showed high expression levels of many TR markers, such as *TEAD4*, *EOMES*, *GATA3*, *ETS2*, *TFAP2A*, *ELF5*, *SMARCA4* (*BRG1*), *CDH3*, *MASH2*, *HSD17B1*, *CYP11A1*, *PPARG*, *ID2*, *GCM1*, *HAND1*, *TDK*, *PAG*, *IFN-τ*, and *THAP11*. The expression of many pluripotency markers, such as *OCT4*, *SOX2*, *NANOG*, and *GDF3*, was lower in bTS cells compared with in vitro-produced blastocysts; however, compared with bovine fetal fibroblasts, the expression of these pluripotency markers was elevated in bTS cells. The DNA methylation status of the promoter regions of *OCT4*, *NANOG*, and *SOX2* was investigated, which were significantly higher in bTS cells (*OCT4* 23.90%, *NANOG* 74.40%, and *SOX2* 8.50%) compared with blastocysts (*OCT4* 8.90%, *NANOG* 34.4%, and *SOX2* 3.80%). In contrast, two promoter regions of *CDX2* were hypomethylated in bTS cells (13.80% and 3.90%) compared with blastocysts (18.80% and 9.10%). The TSC lines that were established in this study may be used either for basic research that is focused on peri-implantation and placenta development or as donor cells for transgenic animal production.

Introduction

Up to now embryonic stem cells (ESCs) have been successfully derived from mice, humans, monkeys, and rats, and trophoblast stem cells (TSCs) have been established from mice [1,2], humans [3,4], rabbits [5], rhesus monkeys [6], and common vole [7]. Despite these successes, it has been notoriously difficult to establish either ESCs or TSCs from ungulates. Although embryonic stem-like cells have been derived from goat [8], cattle [9], and pig [10], these cell lines are morphologically and functionally different from authentic ESCs, which are so-called primed ESCs. Several groups have attempted to harvest TSCs from goat, cattle, and pig, however, only the trophoblast (TR) cell lines have been reported to be obtained from these ungulates [7,11–17]. These TR cell lines continuously grow in culture and show high expression levels of TR cell marker genes. However, the pluripotency and stem cell characteristics of these cells have not been thoroughly examined. These cells likely represent a differentiation stage beyond that of TSCs.

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