

REALIZATION OF SELF-CELL TRANSPLANTATION THROUGH SINGLE BLASTOMERE DERIVED PLURIPOTENT STEM CELLS

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As a possible alternative to the isolation of pluripotent embryonic stem cells from the inner cell mass (ICM) of preimplantation blastocysts, a number of researchers have attempted to derive ES cells from single blastomeres of early pre-compacted embryos. This approach has been viewed favourably, in part for the following reasons: Pre-compacted 8-cell stage embryos were capable of developing normally following the removal of one or two blastomeres. Thus, it allowed the generation of immune-compatible tissue or cells for siblings born from biopsied embryos. Although the ability to derive ES cells from single blastomere represented a crucial advance in stem cell field, its' application is hindered by the low derivation rate. To this end, we have designed strategies to derived ES cell from single blastomeres. In the first strategy, we reconstituted 8 x 1/8 chimeric embryos with eight single blastomeres (1/8), each from different embryos including one eGFP reporter embryo, by microinjection into an empty zona pellucida. Following reconstitution, the chimeric embryos were allowed to form blastocysts and subsequently for ES generation. Overall, we were able to isolate ESC lines from more than 81.3% of the chimeric ICM outgrowths in which 43.8% were contained eGFP+ cells under the 8x1/8 chimera strategy. Fluorescence-activated cell sorting (FACS) revealed that the proportion of eGFP+ cells was varied among the eGFP+ eGFP- chimeric lines. Notably, we found that some of the chimeric lines were predominated (>80%) by the descendants of the single eGFP+ blastomere. These observations indicate that certain 8-cell stage blastomeres may had intrinsic preferentially developmental potential toward pluripotent stem cell or the extrinsic culture condition used for ESC derivation was selectively in favour of the growth of specific blastomere and their progeny toward pluripotnt stem cells. In the second strategy, we generated 1/8+4CPA chimeric embryos by injecting a single GFP-expressing 8 cell stage blastomere into parthenogenetic 4 cell stage embryos. Surprisingly, we found that more than 35% of the single GFP-expressing blastomeres were able to give rise to ES cells after 1/8+4CPA chimeric blastocysts formation and ICM outgrowing. To assess the status of pluripotency, we performed a combination of in vitro and in vivo assays including RT-PCR and immunocytochemistry analysis and teratoma formation test on the single blastomere derived ES cell lines. Immunostaining and RT-PCR showed that these lines expressed the ESC markers Oct-4, Sox2, and SSEA1. In vitro and in vivo differentiation analysis indicated that they were able to give rise to cell types representing all three embryonic germ layers. Through embryo transfer procedures and 1/8+4CPA chimera method for ES cell establishment, we were able to generate pairs of ESC-animal with identical genetic background. Taken together, our results demonstrated that chimera formation by aggregation of 8-cell stage blastomeres each from different embryos is a highly efficient method to isolated isogenic ESC from multiple individuals simultaneously.