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Karyotype Analysis of Parthenote ⇔ Tetraploid Chimeric Blastocysts in Mice*

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ABSTRACT

Parthenote ⇔ tetraploid chimeric embryos were produced by aggregation of a parthenogenetically activated embryo and a tetraploid embryo. The karyotype of these chimeric embryos were observed 48h after aggregation of embryos. Tetraploid embryos were produced by electrofusion at the 2-cell stage, and about 64% of these embryos developed to blastocysts. Parthenogenetic embryos were obtained by ethanol treatment. Eggs with second polar bodies were selected as haploid parthenogenetically activated embryos. The rate of activation was 71%, and 63% of these activated ova developed to 8-cell stage embryos. Thirty-six percent of haploid parthenote ⇔ tetraploid chimeric embryos developed to blastcysts. Chromosomal analysis of chimeric embryos showed that these embryos were consisted of haploid(n) ⇔ diploid(2n), diploid(2n) ⇔ tetraploid(4n), only haploid(n), only diploid(2n) and only tetraploid chromosome constitution.

These results suggested the following three points. Firstly, parthenote ⇔ tetraploid chimeric embryos can develop to blastocysts, secondly, parthenogenetic or tetraploid cell may diploidize spontaneously, and thirdly, diploid cell ratio increased with the numbers of cells per blastocyst.

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INTRODUCTION

Snow[1] reported that living tetraploid mouse basies could be gotten by cytochalasin-B treatment. But, there are no reports that living parthenote was gotten so far. So making chimeras such as tetraploid embryo ⇔ normal diploid embryo, parthenogenetic embryo ⇔ normal diploid embryo, have been studied for extension of survival time in each tetraploid and parthenogenetic embryos[2, 3]. It has also been reported that embryonic tissues derived from tetraploid embryos are extremely underdeveloped. But their extraembryonic tissues can develop, and parthenogenetic cells can contribute to embryonic tissues in chimeras[4, 5, 6].

We expected that tetraploid cells and parthenogenetic cells would rescue each other in chimeric embryos. Therefore, in present study, we investigated the developmental potential of aggregation chimeras which were formed from a tetraploid embryo and a parthenogenetic embryos into blastocyst and their karyotype in mice.

MATERIALS AND METHODS

Parthenogenetic haploid embryos

C57B1/6 females were mated with C3H/He males. The F1 females were superovulated by injection of 5 i. u. of pregnant mare's serum gonadotropin followed by 5 i. u. human chorionic gonadotropin(hCG) 48h later. Mice were sacrificed 17-18hr after hCG treatment, and oviducts were excised. Unfertilized eggs with cumulus cells were removed from their oviducts. These eggs were soaked for in a solution of 7% ethanol in M2 medium for 7min. Five hours later, cumulus cells were removed and eggs with second polar bodies were selected for further culture at 37℃, 5% CO^2 in air.

Tetraploid embryos

ICR females were superovulated and mated with same strain males, and were checked for the vaginal plugs(day 0). On day 1, the pregnant females were sacrificed and the 2-cell embryos were removed from the oviducts. Tetraploid embryos were produced by electrofusion method at 2 times of DC pulse 1.5KV/cm 150μ sec[7]. Tetraploid embryos were selected for further culture.

Production of haploid ⇔ tetraploid chimeric embryos

The zona pellucida was removed by incubation with 0.4% pronase in M2 medium. Then, a 8-cell stage haploid parthenogenetic embryo was aggregated with a 4-cell stage tetraploid embryo in M16 medium containing 0.2% phytohe-magglutinin-P. The aggregated embryos were washed three times, and were cultured in a drop of the M16 medium for 48h.

Karyotype analysis of embryos

Karyotype analysis of chimeric blastocysts was carried out by method of Kamiguchi et al[8].

RESULTS

About 36% chimeric embryos of the aggregates developed to blastocysts 48h after aggregation treatment. There were no significant differences in their development between chimeric embryos and control embryos(Table 1). Aggregated embryos began to unite about 6h after treatment and completelt integrated into an individual embryos 18-24h after treatment.

Table 1. Development of haploid / tetraploid chimeric embryos

Group	Total No. of chimeric embryos examined	No. of embryos developed to blastocysts	Percentage of embryos developed to blastocysts(%)
Control (2n/2n)	10	5	50.0
Chimeric embryos (n/4n)	83	30	36.1

Karyotype analysis of chimeric blastocysts was shown in Table 2. Twelve blastocysts out of total 30 blastocysts had mitotic plates at the time of karyotype analysis. There were no chimeric embryos which had mitotic plates with both haploid cells(n) and tetraploid cells(4n). But, various chimeric embryos which had both haploid cells(n) and diploid cells(2n), both diploid cells(2n) and tetraploid

cells(4n), only haploid cells(n), only diploid cells(2n) and only tetraploid cells(4n), were observed.

The number of cells per blastocyst were shown in Table 3. The chimeric blastocysts have more number of cells than haploid parthenogenetic(n) or tetraploid blastocysts(4n).

Table 2. Karyotype analysis of haploid / tetraploid blastocysts at 48h after aggregation treatment.

Total No. of blastocysts examined	No. of Blastocysys with mitotic plate	No. of blastocysts and their karyotype	
30	12	0	n/4n
		2	n/2n
		2	2n/4n
		3	n
		3	2n
		2	4n

Table 3. Cell counts of blastocysts at 48h after aggregation treatment.

Group	Ploidy	Total No. of blastocysts examined	No. of cells (Mean±S.D.)
Control	2n	13	44.2 ± 9.9
	n	5*	21.0 ± 4.8[a]
	4n	5	15.4 ± 4.8[a]
Chimeric embryos	n/4n	30	54.6 ± 30.5

Values with different superscripts are significantly different each other($p < 0.05$ Student's t-test).
* : Three blastocysts and two 16-cell stage embryos.

Table 4. Cell counts of haploid / tetraploid chimeric blastocysts sorted by size of nucleus.

Group(No. of cells per blastocysts)	Total No. of blastocysts examined	Total No. of cells	Size of nucleus Large : Middle : Small (4n : 2n : n)
Few (16-21)	3	54	0 : 54 : 0
Medium (24-44)	13	406	49 : 306 : 51
			(1 : 6 : 1)
Many (54-115)	14	1,178	93 : 995 : 90
			(1 : 11 : 1)
Total (16-115)*	30	1,638	142 : 1,355 : 141
			(1 : 10 : 1)

* : Chimeric blastocysts were consisted of various number of cells.

Since there were three sizes of nuclei in chimeric blastocysts, we sorted nuclei into three types. The cells which had large, medium and small nuclei were tetraploid, diploid and haploid cells respectively. The nuclear size of thirty chimeric blastocysts was examined in this experiment. The cell counts of three type ploidies were shown in Table 4.

DISCUSSION

About 36% of the chimeric embryos developed to blastocysts in present study. This developmental rate was low compared with another reports[2, 3, 4] in which haploid(n) ⇔ normal diploid(2n), normal diploid(2n) ⇔ tetraploid(4n) chimeric embryos were cultured in vitro. It may be due to that parthenote ⇔ tetraploid chimeric embryos were cultured in present experiment.

Karyotypes of chimeric blastocysts indicated that each tetraploid cells and haploid parthenogenetic cells could live in chimeric blastocysts(Table 2), and chimeric blastocyst contained diploid cells. This suggested that parthenogenetic cells or tetraploid cells diploidized spontaneously in tetraploid ⇔ parthenogenetic chimeric blastocysts. Isino[4] reported that haploid cells derived from parthenotes in haploid parthenote ⇔ fertilized normal embryos on day 9-10, diploidized during the blastocyst or egg cylinder stage. Diploidization may be necessary for survival of mammalian embryos.

Only 12 blastocysts with mitotic plate were observed by the analysis of karyotype and it was not enough to study development of embryos in detail. At pesent, there are no easy methods of examination in karyotype of embryonic cells.

We paid attention to the size of nucleus as a marker of ploidy. We assumed that volume of nucleic acid increase with ploidy of cells. Therefore, we sorted out cells into 3 types in chimeric blastocyst cells by size of nucleus for convenience sake. The cells whose nuclear size were two-times as large as normal one were supposed to be tetraploid. The cells which had a small nucleus were supposed to be haploid. Table 4 indicated that diploid cell counts increased with number of cells per blastocyst, while tetraploid cell counts and haploid cell counts decreased with number of cells per blastocyst. We could not make clear which cell diploidized, tetraploid cell or haploid cell. The proportion of tetraploid and haploid cells of chimeric embryos had to be ratio of 1 to 2 theoretically, but it became same ratio in this experiment. As the number of tetraploid cells in chimeric embryo was a

half of that of haploid cells from the first, haploid cells may diploidize more easily than tetraploid cells. Also, there was a possibility that the tetraploid cells died out or were gradually excluded from chimeric embryos.

As we didn't transfer these chimeric embryos into recipients, extention of survival time by rescueing each other in two different types of cells of chimeric embryos was left unknown.

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