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Review Article

Haploidy of somatic cells in mouse oocyte using somatic cell nuclear transfer

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ABSTRACT Haploidization in somatic cells is the process of reducing the diploid somatic chromosomes to haploid. Several studies have attempted somatic haploidization using oocytes in mice and humans. Some researchers showed partial somatic haploidization, but none observed embryo development. Our study attempted somatic haploidization using the modified somatic nuclear transfer (SCNT) protocol with various combinations of chemicals or proteins in mice. This study induced the proper segregation of somatic homologous chromosomes and full embryo development *in vitro*. Furthermore, somatic haploid embryos established embryonic stem cells and produced live births. The current review summarizes this recent study on the success of somatic haploidization and provides an overview of other related studies on somatic haploidization.

Keywords: chromosomal segregation, homologous chromosome, mice, SCNT, somatic haploidization

INTRODUCTION

Meiosis is the cell division that produces haploid gametes, such as sperms and oocytes, from diploid germ cells (Wang and Pepling, 2021). Meiosis consists of one DNA replication and two nuclear divisions: meiosis I separate pairs of homologous chromosomes and meiosis II separates sister chromatids (Zanders and Malik, 2015). Unlike mitosis, meiosis involves the pairing and recombination of homologous chromosomes (Wilkins and Holliday, 2009). Homologous chromosome pairing is necessary for correct homolog segregation during meiosis (Wang and Pepling, 2021). The inhibition of sister-chromatid segregation in meiosis I and the absence of DNA replication in meiosis II also differ from mitosis (Wilkins and Holliday, 2009).

Somatic haploidization is the process in which the number of chromosomes, such as meiosis, is reduced to a haploid from diploid somatic cells (Nagy and Chang, 2007). This process could create artificial gametes containing the genome of somatic cells, which could be the ultimate opportunity for infertile patients who want genetically related offspring (Zhang et al., 2020).

Until now, several investigators have attempted somatic haploidization. To induce haploidization of the diploid somatic genome, somatic cells were transferred into enucleated oocytes (Tesarik et al., 2021). However, earlier studies have shown abnormal separation and alignment processes in reconstructed chromosomes and limited development of the preimplantation embryos (Fulka et al., 2002; Palermo et al., 2002; Tateno et al., 2003A; Chang et al., 2004; Tesarik et al., 2021). One research group

induced somatic haploidization in the reconstructed oocytes by fertilization using spermatozoon in humans, resulting in the segregation of homologous chromosomes shown in only several chromosomes, and the embryo was not developed (Tesarik et al., 2001).

Recently, one study tried somatic haploidization using somatic cell nuclear transfer with fertilization in mice (Lee et al., 2022). This study demonstrated that the somatic homologous chromosomes were segregated and the somatic haploid (SH)-embryos developed into blastocysts carrying somatic genomes. Further, those embryos established embryonic stem cells (ESCs) and produced offspring (Fig. 1).

The formation of the meiotic spindle–chromosomal complex from somatic chromosomes and the induction of somatic haploidization by fertilization

G0/G1 somatic cells, transferred into enucleated MII oocytes, could form a meiotic spindle-chromosomal complex in SCNT oocytes (Lee et al., 2022). A comparable spindle-chromosomal complex to that in intact metaphase oocytes was observed at 2 hours after SCNT. These SCNT oocytes showed a chromosomal arrangement similar to metaphase I compared to metaphase II. The prometaphase-like or anaphase-like arrangement appeared before or after 2 hours after SCNT.

Reconstructed oocytes were fertilized and then a set of somatic chromosomes was extruded from the cytoplasm to the form of a pseudo-polar body (PPB) (Lee et al., 2022). The remaining somatic and the sperm chromosomes form two nuclei (2PNs) in the zygote. These morphological changes after fertilization (2PN/1PPB) could be indicative of somatic chromosomal segregation. The rate of PPB extrusion was highest in SCNT oocytes resting for 2 hours after SCNT.

This study used the modified SCNT protocol with various combinations of chemicals or proteins, which could assist with chromosome segregation. Fasudil (a ROCK, Rho-associated protein kinase, inhibitor), retinoic acid (RA), and RAD51-Stimulatory Compound 1 (RS-1) were used to improve somatic haploidization. Fasudil treatment might cooperate with the spindle decomposition and regulate microtubule polarity during fertilization (Duan et al., 2014; Yu et al., 2014). RA, which initiates entry into the prophase of meiosis I during oogenesis (Baltus et al., 2006; Nasiri et al., 2011), could make G0/G1 somatic cells enter the prophase in meiosis I because of the hypothesis about the similarity between premature chromosomes from the G0/G1 somatic cell and prophase of meiosis I of the oocyte.

Finally, RS-1, a Rad51 enhancer, is responsible for homologous recombination through homologous centromere coupling and homologous chromosome pairing (Lan et al., 2020), which could support the alignment and segregation of somatic homologous chromosomes. The treatment with these chemicals and proteins increased the rate of 2PN/1PPB formation from 19% to 67%.

Segregation of somatic homologous chromosomes

Our study investigated the segregation of somatic ho-

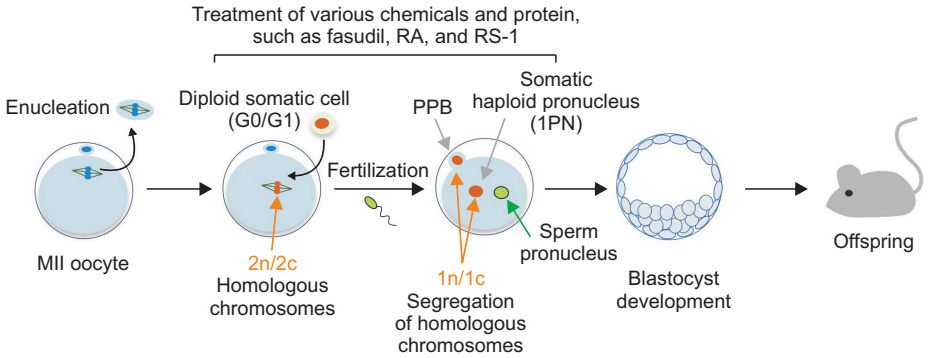

Fig. 1. Induction of somatic haploidization by the mature oocyte in mice. The G0/G1 somatic cell was transferred into the enucleated MII oocyte and the somatic cell-derived spindle was reconstructed. During the resting time after SCNT and fertilization, various chemicals and proteins, such as fasudil, retinoic acid (RA), and RAD51-Stimulatory Compound 1 (RS-1), were treated. Fertilization induced the extrusion of the pseudo-polar body (PPB) and the formation of two pronuclei, one from the somatic nucleus and the other from sperm. Somatic homologous chromosomes were segregated into PPB and somatic haploid embryos. The somatic haploid embryos can develop into blastocysts and produce live offspring.

mologous chromosomes with genetic analysis such as exome sequencing (Lee et al., 2022). This study analyzed PPBs and embryos in somatic haploid zygotes and demonstrated that homologous chromosomes in the SCNT oocytes were segregated randomly after fertilization and generated haploid chromosomes from somatic cells in SH zygotes (Fig. 2).

Prior to this study, the success of chromosome segregation from the somatic cell was debated (Tateno et al., 2003B; Tesarik et al., 2021). The attempt at somatic haploidization using the mature oocytes in humans resulted in the segregation of homologous chromosomes into five chromosomes (Tesarik et al., 2001). Other studies attempted somatic haploidization using immature oocytes in humans and mice (Fulka et al., 2002; Palermo et al., 2002), resulting in less than 1% extrusion of the first polar body and failure of metaphase plates.

Somatic haploidy was also tried using mature oocytes with chemical activation in mice (Tateno et al., 2003A; Tateno et al., 2003B), however, no metaphase-like-array in reconstructed oocytes was seen and the PPB-extruded oocytes failed to present a haploid number of chromosomes. A recent study also used mature oocytes and demonstrated a metaphase-like spindle-chromosomal complex in reconstructed oocytes and the haploid PPB (Lee et al., 2022). This study suggested that the modified SCNT protocol could induce proper chromosome segregation. Proper segregation of homologous chromosomes was observed in all somatic haploid zygotes under this protocol and some zygotes showed proper segregation in all chromosomes.

This study also confirmed that the maternal or paternal alleles of each somatic chromosome existed randomly in somatic haploid embryos, which is a different claim from other studies suggesting that the semi-cloning, the retaining chromosomes of just one parental origin, occurred during somatic haploidization and the success rate of semi-cloning is rare (Tateno et al., 2003B). However, the recent study (Lee et al., 2022) did not consider the semi-cloning, because it was hypothesized that it was not necessary to transmit only just one parental origin in somatic haploid embryos. Therefore, this study focused on the proper segregation of each somatic homologous chromosome, resulting in an average of 76% of the homologous chromosome being properly segregated.

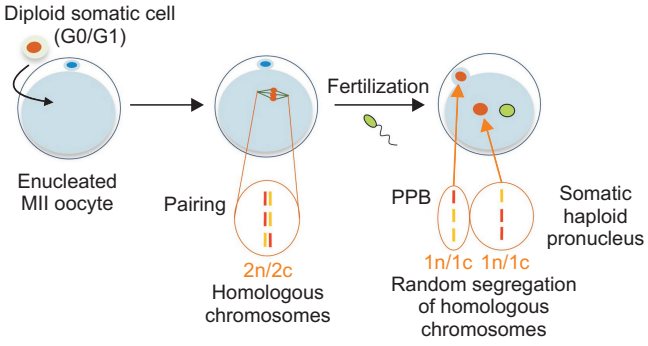

Fig. 2. Random segregation of somatic homologous chromosomes. Diploid G0/G1 somatic nucleus derived-meiotic spindle could consist of 2n/2c state chromosomes. After fertilization, the pseudo-polar body (PPB) and somatic haploid pronucleus are composed of 1n/1c state chromosomes. During the chromosomal segregation, homologous chromosomes were segregated randomly.

Somatic haploid embryos develop into ESCs and offspring

After somatic haploidization by fertilization, the zygotes are able to develop to blastocyst stage and retain somatic chromosomes during embryo development (Lee et al., 2022). This study also established embryonic stem cells (ESCs) (So et al., 2020; Kim et al., 2022) from somatic haploid blastocysts, and these ESCs also contained somatic chromosomes and showed comparable global gene expression to intact ESCs generated from *in vitro* fertilized (IVF) blastocysts. Furthermore, the somatic haploid blastocysts were transferred to recipients (Lee and Kang, 2021) and could produce live offspring.

Even somatic haploid embryos could develop into blastocysts and produce live offspring however, the efficiency was lower compared to regular IVF embryos (Lee et al., 2022). This phenomenon could be explained by the loss of chromosomes in somatic haploid embryos due to failure of chromosome segregation, the limitations of the reprogramming and development in SCNT (Whitworth and Prather, 2010; Matoba and Zhang, 2018), and the discrepancy of the reprogramming cycle between sperm and somatic nucleus.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

The history of attempts to create oocytes with somatic cell nuclei has been controversial. The recent study is the

first to produce live births from somatic haploid embryos, which could be a novel approach to create oocytes carrying somatic genomes (Lee et al., 2022).

Therefore, this approach could be valuable to infertile women who do not have oocytes upon retrieval because there are no techniques to apply to these women to obtain genetically related babies currently. Furthermore, this new technique could be one of the next-generation assisted reproductive technologies along with the technology to generate artificial oocytes from pluripotent stem cells (Hikabe et al., 2016; Hamazaki et al., 2021).

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