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LINE-1 of Evidence for Fetal Oocyte Attrition by Retrotransposon

Shinichiro Chuma[1,*]
[1]Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
*Correspondence: schuma@frontier.kyoto-u.ac.jp
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Fetal oocytes in mammals undergo extensive apoptosis during development. In this issue of *Developmental Cell*, Malki et al. (2014) provide insight into how and why such massive oocyte loss occurs through the demonstration that the expression level of LINE-1 retrotransposon defines the survival threshold and thus viability of fetal oocytes.

During oocyte development, only a limited fraction of the initial pool of fetal oocytes survives through to postnatal follicular growth and ovulation; most of the developing oocytes are lost via programmed cell death during differentiation and maturation. While such developmental cell death is widely observed in various somatic cell lineages (for example, in the nervous system and the limb bud), germ cell death is unique in that any selection occurring among the cell population may affect the genome and epigenome of the next generation. Programmed cell death of oocytes is first evident in female embryos just after primordial germ cells enter meiotic prophase I to become oocytes. Starting from early meiosis, fetal oocyte number dramatically decreases to one-third to one-fourth of the original pool by the time of birth (Tilly, 2001; Pepling, 2006). Previous genetic studies have shown that such massive elimination of early meiotic oocytes, often termed fetal oocyte attrition, occurs via apoptotic pathways. However, the primary trigger of fetal oocyte attrition is unknown. Whether extrinsic stimuli (such as growth factor deprivation or niche competition) transmit the death signal or whether intrinsic mechanisms (such as cellular responses to DNA damage or other stresses) activate checkpoint-induced apoptosis have also long been a matter of debate.

One distinctive feature of fetal oocytes is sustained genome-wide DNA hypomethylation. During germline development, genome CpG methylation marks are globally erased during primordial germ cell formation. De novo DNA methylation is then reestablished in fetal prospermatogonia in the male, whereas in the female, global hypomethylation is maintained in fetal oocytes, with de novo DNA methylation being imposed after birth (Schaefer et al., 2007). The global erasure of genome DNA methylation in the germline is considered essential to reprogramming epigenetic information toward gametogenesis and embryogenesis, but this methylation loss also poses a potential threat to the germline genome by reanimating mobile transposable elements. Indeed, both prospermatogonia and fetal oocytes express detectable levels of transposons, although the developmental consequences are unknown.

In this issue of *Developmental Cell*, Malki et al. (2014) now connect LINE-1 retrotransposon activity to fetal oocyte attrition in mice. LINE-1 is the most abundant class of retrotransposons, comprising about 20% of the mammalian genome, with approximately 10,000 copies being full length in mice (Goodier and Kazazian, 2008). The authors first examined Maelstrom null fetal ovaries to investigate a possible correlation between increased LINE-1 expression and oocyte survival, as well as meiotic progression. Maelstrom, an evolutionarily conserved HMG domain-containing protein preferentially expressed in the germline, is essential for spermatogenesis in mice and suppresses retrotransposons through the regulation of germline-specific piwi-interacting small RNA (piRNA) biogenesis (Soper et al., 2008). The piRNA system primarily functions in the male germline in mice, and its role in female reproduction remains unclear. One recent study reported that retrotransposon expression is increased in fetal oocytes of several piRNA pathway mutants but that the mutant females are fertile with no apparent oogenesis defects (Lim et al., 2013).

In the current study, Malki et al. (2014) found that Maelstrom null ovaries show increased fetal oocyte attrition and diminished oocyte reserve. The mutant females have a shortened reproductive lifespan, made evident by backcrossing Maelstrom null mice to an inbred genetic background. LINE-1 retrotransposon expression was significantly upregulated in Maelstrom null fetal oocytes (2- to 3-fold increase, on average, relative to controls), while expression of IAPs, another major class of retrotransposons, was not detectable in either Maelstrom null or wild-type oocytes. The authors then quantified in detail LINE-1 ORF1 protein, one of the two proteins encoded by LINE-1 (the other ORF2 protein is much less abundant and very difficult to detect) by immunofluorescence in developing oocytes at embryonic day 15 (E15), E18, and postnatal day 2 (P2). The surprise from this immunohistological analysis is that each oocyte expresses a highly variable amount of LINE-1 ORF1. It appeared that LINE-1 ORF1 levels in early fetal oocytes (at E15) set the threshold for the survival of later-stage oocytes (at E18 and P2). That is, assuming that early fetal oocytes that experience excessive LINE-1 expression above a certain threshold are destined for apoptotic elimination, then the survivor number from LINE-1 selection nicely matched what would be predicted as the number of viable oocytes at later stages. Consistent with the involvement of LINE-1, whose transposon activity induces genome DNA damage, Maelstrom null oocytes associated with excessive LINE-1 expression exhibit enhanced DNA strand breaks

and synapsis failure of homologous chromosomes during meiotic prophase I, which can activate checkpoint pathways and trigger cell death.

To further investigate a more direct consequence of LINE-1 overexpression, the authors employed an inducible transgenic mouse system of LINE-1 and again observed a significant correlation between the elevated levels of LINE-1 expression and reduced survival rates of fetal oocytes, together with increased meiotic defects, including unrepaired DNA damage and asynapsis. Because a single LINE-1 transgene induces only a moderate increase in total LINE-1 expression in the presence of more than 10,000 full-length endogenous LINE-1 copies in the genome, the phenotypes observed by this transgenic experiment were somewhat milder than those seen in Maelstrom null mutants. However, this gain-of-function experiment of LINE-1 corroborated the idea that excessive LINE-1 expression directly enhances fetal oocyte attrition, possibly by inducing meiotic defects.

Given these observations, can inhibition of LINE-1 activity alleviate fetal oocyte attrition? Side stepping the challenges of knocking out numerous copies of LINE-1 in the genome or RNAi knockdown of LINE-1, whose widespread sequences in transcriptome would likely induce off-target effects, Malki et al. (2014) made use of the nucleoside analog azidothymidine (AZT), which inhibits reverse transcriptase activity and thus retrotransposon integration. Administration of AZT to pregnant female mice resulted in a transient but remarkable block of fetal oocyte attrition in both wild-type and Maelstrom null genetic backgrounds. Almost all of AZT-treated wild-type oocytes (more than 90%) survived between E15 and E18. The viability of AZT-treated Maelstrom null early oocytes also recovered 2- to 3-fold between E15 and E18 relative to untreated mutants. The numbers of later oocytes (at P2) dropped to almost similar levels in both AZT-treated and untreated wild-type ovaries, whereas in Maelstrom null ovaries, AZT treatment still significantly alleviated severe oocyte attrition at P2. This transient—but not stable—effect of AZT is attributable to the endonuclease activity of LINE-1 in the presence of AZT, which can induce DNA strand breaks as the initial step of target-primed retrotransposition before reverse transcription and integration. Indeed, AZT-treated wild-type fetal oocytes with increased survival rate exhibited elevated DNA damage together with meiotic asynapsis. A possible explanation for this observation is that reverse transcriptase-dependent intermediates of LINE-1 retrotransposition exert a detrimental effect on early oocyte survival and meiosis quality, whereas DNA damage induced by LINE-1 endonuclease activity triggers oocyte loss at later stages. Together, these results provide a proof of principle that fetal oocyte attrition can be modulated by controlling LINE-1 activity.

With the discoveries reported in this study, many new questions arise. For example, it is still unclear whether LINE-1 expression is intrinsically regulated as the primary trigger of fetal oocyte attrition or whether extrinsic factors (such as cellular stresses or nutritional environment) activate LINE-1 expression as a downstream cell death mediator of such extracellular cues. With respect to the latter notion, it is well established that retrotransposon expression is upregulated in response to various cellular stimuli, such as heat shock, DNA damage, and certain chemical treatments in somatic cells. Whether a similar mechanism operates to induce LINE-1 in fetal oocytes needs future investigation. It would also be worth examining whether retrotransposon-induced developmental cell death operates in somatic cell lineages or whether such a mechanism is specific to oocytes or germline cells in which extensive cellular suicide is worth the cost to protect the genomic information of the next generation. From a practical point of view, if oocyte attrition can be suppressed by artificially shutting down retrotransposon activity, then this may present a possible tool for extending female reproductive lifespan without sacrificing oocyte quality.

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