Zidovudine Inhibits Telomere Elongation, Increases the Transposable Element LINE-1 Copy Number and Compromises Mouse Embryo Development.

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Research Article

Keywords: Zidovudine, telomere, Long Interspersed Nuclear Element 1, mouse oocytes, mouse embryos

Posted Date: August 4th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-696789/v1>

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Version of Record: A version of this preprint was published at Molecular Biology Reports on October 20th, 2021. See the published version at <https://doi.org/10.1007/s11033-021-06788-x>.

Abstract

Purpose

Millions of pregnant, HIV-infected women take reverse transcriptase inhibitors, such as zidovudine (AZT), during pregnancy. Reverse transcription plays important roles in early development, including regulation of telomere length (TL) and activity of transposable elements (TE). So we evaluated the effects of AZT on embryo development, TL, and copy number of an active TE, Long Interspersed Nuclear Element 1 (LINE-1), during early development in a murine model.

Design:

Experimental study

Methods

In vivo fertilized mouse zygotes from B6C3F1/B6D2F1 mice were cultured for 48h in KSOM with no AZT (n = 45), AZT 1 μ M (n = 46) or AZT 10 μ M (n = 48). TL was measured by single-cell quantitative PCR (SC-pqPCR) and LINE-1 copy number by qPCR. The percentage of morulas at 48 hrs, TL and LINE-1 copy number were compared among groups.

Results

Exposure to AZT 1 μ M or 10 μ M significantly impairs early embryo development. TL elongates from oocyte to control embryos. TL in AZT 1 μ M embryos is shorter than in control embryos. LINE-1 copy number is significantly lower in oocytes than control embryos. AZT 1 μ M increases LINE-1 copy number compared to oocytes controls, and AZT 10 μ M embryos.

Conclusion

AZT at concentrations approaching those used to prevent perinatal HIV transmission compromises mouse embryo development, prevents telomere elongation and increases LINE-1 copy number after 48 hours treatment. The impact of these effects on the trajectory of aging of children exposed to AZT early during development deserves further investigation.

Introduction

The use of antiretroviral drugs during pregnancy dramatically reduced the risk of perinatal transmission of human immunodeficiency virus (HIV) to less than 1% [1]. However, concerns remain about the safety

of antiretroviral agents during pregnancy, and careful assessment of the risks associated with specific medications is needed to conduct treatment decisions. Zidovudine, a reverse transcriptase inhibitor (RTi), is an antiretroviral agent often used to treat HIV during pregnancy. However, it is no longer a preferred component of first-line treatment, mainly because of programmatic reasons unrelated to safety [2]. However, until now, millions of pregnant, HIV-infected women have taken AZT during pregnancy to prevent perinatal transmission of HIV [1].

Telomeres are repetitive sequences of non-coding DNA associated with specialized proteins known as shelterin complex that protect telomeres from being recognized by DNA damage surveillance and processed by DNA repair pathways [3]. Telomere degrades with each replication due to inability of DNA polymerase enzyme to complete synthesizing the chromosomal ends. This fact limits the number of cell divisions that a cell can tolerate and, if telomeres reach a critical length senescence and programmed cell death can occur [4]. During the earliest stages of preimplantation embryo development, telomeres undergo extensive lengthening, both from telomerase-independent and telomerase-dependent mechanisms [5]. In mouse embryos, the most dramatic telomere lengthening takes place before implantation [6]. In cleavage stage embryos, telomeres elongate in kilo base increments, independent of telomerase and sperm, via a double-strand DNA-based mechanism involving telomere sister chromatid exchange denominated alternative lengthening of telomeres (ALTs) [5]. Some cancer cells also elongate their telomeres through ALTs [7].

LINE-1 (long interspersed nuclear elements) retrotransposons constitute a large family of retrotransposable elements, accounting for 17% of the human genome [8]. They encode proteins required for their own mobilization, including a reverse transcriptase (RT) enzyme highly expressed in mouse embryos and mouse and human cancer cells and repressed in somatic differentiated healthy cells [9]. Reverse transcription takes place during early murine embryo development, yielding increased LINE-1 copy number during preimplantation development, which also occurs in tumor progression. Indeed, RT inhibition, either by pharmacological inhibitors or by RNA interference (RNAi) to LINE-1 elements arrests development at the two or four-cell stages, accompanied by significant alterations of gene expression profiles compared to control embryos [10]. RT inhibition irreversibly arrests embryo development, reduces cancer cell proliferation, promotes differentiation, antagonizes tumor growth, and causes global reprogramming of transcription profiles [9].

Intriguingly, human tumor-derived ALT cell lines express a LINE-1 (L1) retrotransposon, which suggests its participation in telomere maintenance, possibly by a «slippage» mechanism of telomeric DNA synthesis [11]. Moreover, suppression of the L1 encoded reverse transcriptase activity using an antisense strategy, or treatment of ALT cells with AZT induces progressive telomere loss, arrest in G2-phase of the cell cycle, and, eventually, cancer cell death [11]. Little is known about the effects of AZT on TL and LINE-1 copy number of cleavage stage embryos.

AZT shortens telomeres in immortalized human T- and B-lymphocyte cell lines and induces progressive telomere loss in ALT cells, and some species employ transposable elements (TE) to cap chromosome

ends. Thus, this study aims to investigate the effects of AZT on development, TL, and LINE-1 copy number during early development using a murine model.

Materials And Methods

Mouse Samples and experimental groups

A pilot study used cryopreserved metaphase II oocytes and one-cell *in vivo* fertilized embryos from B6C3F1/B6D2F1 mice (Embryotech Laboratories, Inc., Wilmington, MA). Oocytes and one-cell embryos were thawed by removing them from liquid nitrogen container then exposing them to room temperature for 2 minutes. Each straw was moved to a 37°C water heated container for 1 minute before cutting the straw and transferring the samples to a Petri dish containing M2 culture medium (EmbryoMax® M2 Medium, 1X). Oocytes and embryos were washed three times in M2 medium. MII oocytes were individually stored in sterile PCR microtubes containing 1 µL 0.1 % phosphate buffered saline (PBS; Invitrogen, Grand Island, NY, USA) - polyvinylpyrrolidone (PVP, Sigma-Aldrich, St. Louis, MO, USA) in -80 °C for further analysis.

One-cell mouse embryos were *in vitro* cultured for 48h in potassium simplex optimized medium (KSOM, Merck Millipore, Germany) under mineral oil at 37°C in 6% CO₂ in humidified air with no AZT (control group, n = 45), or AZT 1 µM (n = 46) or 10 µM (n = 48) for 48 hours. After 24 or 48 hours in culture, embryo developmental stage was evaluated. Embryos still viable after 48 hours in culture were individually stored in sterile PCR microtubes containing 1 µL 0.1 % PBS/PVP in -80 °C for further analysis.

Oocyte single-cell genomic DNA and embryo genomic DNA were obtained by adding 1 μ L lysis buffer 2 $\times$ (100 mM Tris $\cdot$ HCl pH 7.4, 300 mM NaCl, 0.8 mM EDTA, 2 % (*vol/vol*) Nonidet P-40, and 5 mM DTT) into PCR microtubes and heated at 75°C for 15 min. and cool down at room temperature. Oocyte single-cell genomic DNA and embryo genomic DNA were individually stored at -20°C until performing single-cell quantitative PCR (SC-pqPCR) to evaluate mean telomere length and qPCR to determine LINE-1 copy number.

Telomere length assay in individual oocytes and mouse embryos

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reverse primer (10 μ M, m36B4-F: 5'ACTGGTCTAGGACCCGAGAAG'3; m36B4-R: 5'TCAATGGTGCCTCTGGAGATT'3), and water to a 40 μ L final volume. Thermal cycler reaction conditions were 95°C for 10 minutes followed by 40 cycles of data collection at 95°C for 15 seconds, 60°C annealing for 30 seconds and 72°C extend for 30 seconds along with 80 cycles of melting curve from 55°C to 95°C.

PCR products were purified following the protocol of the purification kit (DNA clean and concentrator-5; Zymo Research, USA) and were eluted in 64 μ L of double distilled water. Finally, the purified products from each individual oocyte were aliquoted into each well of a 96-well plate to perform the real-time PCR. Each sample was run in triplicate along with a target-specific no-template control (NTC). Positive controls were allocated to random wells on random plates to assure plate-to-plate concordance and DNA from HeLa cells (New England Biosciences, Ipswich, MA, USA) were serially diluted (20, 4, 0.8, 0.16, and 0.032 ng/ μ L) as a reference for standard curve calculation. After thermal cycling was completed, the CFX manager software was used to generate standard curves and Ct values for telomere signals and reference gene (m36B4) signals. To ensure high reproducibility of samples, only assays with real-time PCR efficiencies between 95 and 105 % and intra-assay coefficient of variation (CoV) less than 1 % were included in analysis.

LINE-1 copy number assay in individual oocytes and mouse embryos

LINE-1 copy number was calculated by comparing the values of LINE-1 (T) and mB1 as a reference (R). Pre-PCR was performed as described above. Each reaction was set up with 4 μ L 10xPCR buffer, 4 μ L 2.5 mM dNTP, 0.25 μ L DNA polymerase, 1 μ L each of LINE-1 forward and reverse primer (10 μ M, LINE-1F: GAACCAAGACCACTCACCATCA; LINE-1 R: CCCTGGACTGGGCGAAGT), 1 μ L each of reference genes forward and reverse primer (10 μ M, mB1-F: GCACCTTTAATCCCAGCAC e mB1-R: TGAGACAGGGTTTCTCTGTA), and water to a 40 μ L final volume. Thermal cycler reaction conditions were set at 94°C for 5 minutes followed by 15 cycles of 94°C for 30 seconds, 60°C annealing for 30 seconds and extension at 72°C for 30 seconds, with a final extension for 10 minutes at 72°C. PCR products were purified following the protocol of the purification kit (Agencourt AMPure XP beads) and were eluted in 50 μ L of double distilled water.

Finally, purified products from each individual sample were aliquoted into each well of a 96-well plate to perform the real-time PCR. Each reaction included 10 μ L 2 $\times$ SYBR Green mix (Bio-Rad), 1 μ L each of 10 μ M forward and reverse primers, 4 μ L molecular-filter water and 5 μ L genomic DNA to yield a 20- μ L reaction. DNA samples were placed in 96-well plate for LINE-1 primers and reference gene primers, respectively. A Bio-Rad thermal cycler (CFX system test) was used with reaction conditions of 95°C for 10 minutes followed by 40 cycles of data collection at 95°C for 15 seconds, 60°C annealing and extension for 1 minute. Each sample was run in triplicate along with a target-specific non-template control (NTC). DNA from mouse embryonic stem cells was used as positive control in each plate to assure concordance between plates. After thermal cycling was completed, the CFX manager software was used to generate standard curves and Ct values for LINE-1 and reference gene (mB1). To ensure high reproducibility of

samples, only assays with real-time PCR efficiencies between 95 and 105 % and intra-assay coefficient of variation (CoV) less than 1 % were included in analysis.

Statistical analysis

The percentage of morula stage embryos at 48 hours was compared among groups by Fisher's Test. TL and LINE-1 copy number were compared by Kruskal Wallis' Test and Dunn's Post-Test. A *p* value less than 0.05 were considered significant. Statistics were performed on SPSS 20.0 software (IBM SPSS Software, Armonk, NY, USA), and graphs were plotted using GraphPad Prism 5 software (GraphPad Software, La Jolla, CA, USA).

Results

Effect of AZT on embryo development

To evaluate the effects of AZT on mouse embryo development, *in vivo* fertilized mouse zygotes were cultured for 48h in KSOM with no AZT - control (n = 45), AZT 1 μ M (n = 46) or AZT 10 μ M (n = 48), and the percentage of morulas at 48 hrs was compared among the three groups. Exposure to 1 μ M or 10 μ M AZT significantly impaired embryonic development (controls – 86.7% morula versus AZT 1 μ M – 63% morula versus AZT 10 μ M – 33.3% morula) ($p < 0.01$) (Table 1).

Effect of AZT on telomere length and LINE-1 copy number

As previously reported, telomeres robustly elongate during early embryonic development: mean TL of embryos in the control group (n = 17) significantly exceeded that of oocytes in metaphase II (n = 28; $p = 0.0014$). AZT at both concentrations prevented telomere elongation: TL of embryos cultured for 48 hours with 1 μ M and 10 μ M AZT was not statistically different from MII oocyte TL ($p = 0.1$ and 0.8 , respectively). TL in embryos cultured for 48 hours with 1 μ M AZT was significantly shorter than that of embryos cultured without 1 μ M AZT ($p = 0.04$). TL in embryos cultured for 48 hours with 10 μ M AZT ($p = 0.05$) did not differ than those cultured in control media. No significant difference was observed between TLs of embryos cultured for 48 hours with 1 μ M AZT or 10 μ M AZT (Tables 2 and 3).

As previously reported, LINE-1 copy number increases during early development: LINE-1 copy number in control embryos (n = 21), AZT 1 μ M (n = 21), and AZT 10 μ M (n = 24) significantly exceeded that in metaphase II oocytes (n = 28; <0.001). AZT 1 μ M promoted a significant increase in LINE-1 copy number: LINE-1 copy number was significantly higher in embryos exposed to AZT 1 μ M compared to oocytes ($p = 0.001$), control embryos ($p = 0.026$), and embryos cultured in AZT 10 μ M ($p = 0.0048$) (Table 3).

Discussion

Antiretroviral therapy generally has been considered safe to infants, though many antiretrovirals, such as AZT, cross the placenta during pregnancy, exposing fetuses to it in utero [13]. Here we demonstrate that

AZT at 1 μ M or 10 μ M concentrations significantly impairs embryonic development, consistent with previous studies [14–16]. In addition, this is the first paper to report that AZT prevents telomere elongation during preimplantation embryo development. Based on its significant inhibition of telomere elongation during early development, we cannot rule out the possibility AZT may influence telomere length and hence longevity. Telomere attrition promotes increased risk of morbidity and mortality [17, 18] and increased risks of ageing-related diseases [19]. Telomere attrition is a normal biological event as adults' age, but the impact of starting life with attenuated telomeres begs further study.

AZT, a reverse-transcriptase inhibitor (RTi), blocks LINE-1 RT activity in vitro [20, 21] and in cell-based retrotransposition assays [22, 23]. Premature silencing of LINE-1 decreases chromatin accessibility in mouse embryos, whereas prolonged activation prevents the gradual chromatin compaction that occurs naturally in developmental progression [21]. Also, culturing TALE-L1-VP64 embryos in the presence of AZT promotes arrest, indicating that the developmental arrest elicited after prolonging LINE-1 transcription is independent of RT activity and therefore of retrotransposition [21]. Here we show that AZT at 1 μ M after 48 hours of embryo culture significantly increases LINE-1 copy number compared to MII oocytes, control embryos, and embryos cultured in AZT 10 μ M. Interestingly, LINE 1 copy number in embryos cultured in at 10 μ M AZT does not significantly differ from controls and is significantly lower than that of embryos cultured in AZT 1 μ M. Recently, Banuelos-Sanchez et. al. 2019, successfully decreased retrotransposition of human LINE-1 retrotransposon in HeLa cells with AZT at 5 μ M and 25 μ M concentrations.

Why LINE-1 copy number in embryos cultured at 10 μ M AZT did not differ from control group and was significantly lower than embryos cultured 1 μ M AZT remains unclear. Perhaps higher concentrations of AZT exert effects on a broader array of reverse transcriptases than lower doses. Three different reverse transcriptases are encoded by the human and mouse genomes: i) telomerase, ii) RT encoded by non-long terminal repeat (non-LTR) retrotransposons such as LINE-1 and iii) RT encoded by retroviral elements such as LTR retrotransposons and endogenous retroviruses [24–27]. AZT is a potent but not selective RTi- it inhibits not only the LINE-1 RT, but also mouse ERV RT [28].

The LINE-1 cycle begins with transcription, export of mRNA, and translation of LINE-1 proteins, which are essential for completing retrotransposition [29, 30]. An alternative mechanism, called endonuclease-independent (ENi) retrotransposition or non-classical L1 insertion (NCLI), initiates at pre-existing DNA lesions, without the need for endonuclease cleavage [31–33]. This pathway can lead to retrotransposition at telomeres [34]. LINE-1 uses dysfunctional telomeres as integration substrates for ENi retrotransposition [34]. RT activity is needed in both pathways for complete retrotransposition.

After fertilization, to initiate development, gametes are reprogrammed to become totipotent. Approximately half of the mammalian genome consists of repetitive elements, including retrotransposons, some of which are transcribed after fertilization. Studies indicate that LINE-1 activation is not a mere side effect of epigenetic reprogramming but rather plays an important role in shaping the chromatin landscape of the early embryo [21]. LINE-1 retrotransposon activation taking place soon after fertilization contributes to

genome rearrangement and to the evolution of individual genotypes [24]. The mechanism by which perturbation of LINE-1 retrotransposition by AZT promotes increased LINE-1 copy number and inhibits telomere elongation merits further investigation.

Conclusions

Here we demonstrate that AZT compromises mouse embryo development, prevents telomere elongation and increases LINE-1 copy number at the 1 μ M AZT concentration over 48 hours.

AZT increases LINE-1 copy number and inhibits telomere elongation in preimplantation embryos after just 48 hours treatment. Since short telomeres in adults have been associated with increased morbidity and mortality, as well as aging-related diseases our study highlights the importance of investigating the impact of these effects on the trajectory of aging of children exposed in uterus to AZT. Further research is needed to understand the impact of in utero exposure to AZT on retrotransposons and telomeres later in development.

Declarations

Acknowledgements

This work was supported by Scholarship from FAPESP, Brazil (Process 2015/21907-0), Coordination for the Improvement of Higher Education Personnel (CAPES) - Finance Code:001 (process n. 88887.371487/2019-00) and the Stanley H. Kaplan Fund of the NYU Grossman School of Medicine.

Conflict of interest

The authors declared no conflict of interest.

Institutional Review Board

Institutional Review Board approval was not necessary because we used cryopreserved metaphase II oocytes and one-cell in vivo fertilized embryos from B6C3F1/B6D2F1 mice bought from Embryotech Laboratories (Inc., Wilmington, MA).

Authors Agreement

All authors, verify that the submitted material has not been published and is not currently submitted for publication elsewhere. We agree (i) to the inclusion of our names as authors on the manuscript; (ii) to the order in which our names are shown on the title page of the manuscript; (iii) that all persons listed as authors fulfill the International Committee of Medical Journal Editors (ICMJE) criteria for authorship; and (iv) that no persons who fulfill the ICMJE criteria for authorship have been omitted from the author list. All authors consent for the publication of our article in Molecular Biology Reports if approved.

Availability of data and material

All authors declare data and material are available for consultation.

References

1. Fowler MG, Qin M, Fiscus SA, Currier JS, Flynn PM, Chipato T et al (2016) Benefits and Risks of Antiretroviral Therapy for Perinatal HIV Prevention. *N Engl J Med* 375:1726–1737
2. W.H.O (2016) Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. World Health Organization
3. Palm W, de Lange T (2008) How shelterin protects mammalian telomeres. *Annu Rev Genet* 42:301–334
4. Counter CM, Avilion AA, LeFeuvre CE, Stewart NG, Greider CW, Harley CB et al (1992) Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity. *EMBO J* 11:1921–1929
5. Keefe DL. Telomeres (2016) Reproductive Aging, and Genomic Instability During Early Development. *Reprod Sci* 23:1612–1615
6. Liu L, Bailey SM, Okuka M, Muñoz P, Li C, Zhou L et al (2007) Telomere lengthening early in development. *Nat Cell Biol* 9:1436–1441
7. Gaspar TB, Sá A, Lopes JM, Sobrinho-Simões M, Soares P, Vinagre J. Telomere Maintenance Mechanisms in Cancer. *Genes (Basel)*. 2018; 9
8. Brouha B, Schustak J, Badge RM, Lutz-Prigge S, Farley AH, Moran JV et al (2003) Hot L1s account for the bulk of retrotransposition in the human population. *Proc Natl Acad Sci U S A* 100:5280–5285
9. Spadafora C (2015) A LINE-1-encoded reverse transcriptase-dependent regulatory mechanism is active in embryogenesis and tumorigenesis. *Ann N Y Acad Sci* 1341:164–171
10. Beraldi R, Pittoggi C, Sciamanna I, Mattei E, Spadafora C (2006) Expression of LINE-1 retroposons is essential for murine preimplantation development. *Mol Reprod Dev* 73:279–287
11. Bondarev IE, Khavinson VK (2016) [Suppression of alternative telomere lengthening in cancer cells with reverse transcriptase inhibitors]. *Adv Gerontol* 29:218–221
12. Wang F, Pan X, Kalmbach K, Seth-Smith ML, Ye X, Antunes DM et al (2013) Robust measurement of telomere length in single cells. *Proc Natl Acad Sci U S A* 110:E1906–E1912
13. Gillet JY, Garraffo R, Abrar D, Bongain A, Lapalus P, Dellamonica P (1989) Fetoplacental passage of zidovudine. *Lancet* 2:269–270
14. Toltzis P, Marx CM, Kleinman N, Levine EM, Schmidt EV (1991) Zidovudine-associated embryonic toxicity in mice. *J Infect Dis* 163:1212–1218
15. Toltzis P, Mourton T, Magnuson T (1993) Effect of zidovudine on preimplantation murine embryos. *Antimicrob Agents Chemother* 37:1610–1613

16. Sieh E, Coluzzi ML, Cusella De Angelis MG, Mezzogiorno A, Florida M, Canipari R et al (1992) The effects of AZT and DDI on pre- and postimplantation mammalian embryos: an in vivo and in vitro study. *AIDS Res Hum Retroviruses* 8:639–649
17. Cawthon RM, Smith KR, O'Brien E, Sivatchenko A, Kerber RA (2003) Association between telomere length in blood and mortality in people aged 60 years or older. *Lancet* 361:393–395
18. Wilbourn RV, Moatt JP, Froy H, Walling CA, Nussey DH, Boonekamp JJ. The relationship between telomere length and mortality risk in non-model vertebrate systems: a meta-analysis. *Philos Trans R Soc Lond B Biol Sci.* 2018; 373
19. Aviv A, Shay JW. Reflections on telomere dynamics and ageing-related diseases in humans. *Philos Trans R Soc Lond B Biol Sci.* 2018; 373
20. Dai L, Huang Q, Boeke JD (2011) Effect of reverse transcriptase inhibitors on LINE-1 and Ty1 reverse transcriptase activities and on LINE-1 retrotransposition. *BMC Biochem* 12:18
21. Jachowicz JW, Bing X, Pontabry J, Bošković A, Rando OJ, Torres-Padilla ME (2017) LINE-1 activation after fertilization regulates global chromatin accessibility in the early mouse embryo. *Nat Genet* 49:1502–1510
22. Xie Y, Rosser JM, Thompson TL, Boeke JD, An W (2011) Characterization of L1 retrotransposition with high-throughput dual-luciferase assays. *Nucleic Acids Res* 39:e16
23. Kubo S, Seleme MC, Soifer HS, Perez JL, Moran JV, Kazazian HH et al (2006) L1 retrotransposition in nondividing and primary human somatic cells. *Proc Natl Acad Sci U S A* 103:8036–8041
24. Mueller C, Aschacher T, Wolf B, Bergmann M (2018) A role of LINE-1 in telomere regulation. *Front Biosci (Landmark Ed)* 23:1310–1319
25. Richardson SR, Doucet AJ, Kopera HC, Moldovan JB, Garcia-Perez JL, Moran JV (2015) The Influence of LINE-1 and SINE Retrotransposons on Mammalian Genomes. *Microbiol Spectr* 3:MDNA3–M0061
26. Sciamanna I, Vitullo P, Curatolo A, Spadafora C (2009) Retrotransposons, reverse transcriptase and the genesis of new genetic information. *Gene* 448:180–186
27. Stocking C, Kozak CA (2008) Murine endogenous retroviruses. *Cell Mol Life Sci* 65:3383–3398
28. Banuelos-Sanchez G, Sanchez L, Benitez-Guijarro M, Sanchez-Carnerero V, Salvador-Palomeque C, Tristan-Ramos P et al. Synthesis and Characterization of Specific Reverse Transcriptase Inhibitors for Mammalian LINE-1 Retrotransposons. *Cell Chem Biol.* 2019; 26: 1095 – 109.e14
29. Kulpa DA, Moran JV (2006) Cis-preferential LINE-1 reverse transcriptase activity in ribonucleoprotein particles. *Nat Struct Mol Biol* 13:655–660
30. Wei W, Gilbert N, Ooi SL, Lawler JF, Ostertag EM, Kazazian HH et al (2001) Human L1 retrotransposition: cis preference versus trans complementation. *Mol Cell Biol* 21:1429–1439
31. Sen SK, Huang CT, Han K, Batzer MA (2007) Endonuclease-independent insertion provides an alternative pathway for L1 retrotransposition in the human genome. *Nucleic Acids Res* 35:3741–3751

32. Morrish TA, Gilbert N, Myers JS, Vincent BJ, Stamato TD, Taccioli GE et al (2002) DNA repair mediated by endonuclease-independent LINE-1 retrotransposition. *Nat Genet* 31:159–165
33. Viollet S, Monot C, Cristofari G (2014) L1 retrotransposition: The snap-velcro model and its consequences. *Mob Genet Elements* 4:e28907
34. Morrish TA, Garcia-Perez JL, Stamato TD, Taccioli GE, Sekiguchi J, Moran JV (2007) Endonuclease-independent LINE-1 retrotransposition at mammalian telomeres. *Nature* 446:208–212

Tables

Table 1

Development of mouse embryos from in vivo fertilized one cell embryos cultured for 48 hours in KSOM without AZT (control), AZT 1 μ M and AZT 10 μ M.

Group	Number of one cell mouse embryos cultured	Number of 2 cells embryos (%) after 24 hours in culture	Number of morulas (%) after 48 hours in culture
Control Control	45	43 (95,6) ^a	39 (86,7) ^a
AZT 1 μ M	46	43 (93,5) ^a	29 (63,0) ^b
AZT 10 μ M	48	46 (95,8) ^a	16 (33,3) ^c

Note. Different letters in the same column indicate a significant difference ($p < 0.01$, Fisher's test).

Table 2

Mean telomere length of metaphase II mouse oocytes and embryos obtained from in vivo fertilized one cell embryos cultured for 48 hours in KSOM without AZT (control), AZT 1 μ M and AZT 10 μ M.

Group	Number	Median	Lower Quartile	Upper Quartile	P value
MII oocyte	28	1.00 ^a	0.58	1.78	0,029
Control	17	1.56 ^{a, b}	1.41	2.19	
AZT 1 μ M	21	1.43 ^b	1.43	1.83	
AZT 10 μ M	20	1,57	1.37	1.84	

Note. P value according to Kruskal Wallis' test. Similar letters in the same column indicate a significant difference ($p < 0.05$) by Dunn's post-test. ^a: $p = 0.01$, ^b: $p = 0.04$

Table 3

LINE-1 copy number of metaphase II mouse oocytes and embryos obtained from in vivo fertilized one cell embryos cultured for 48 hours in KSOM without AZT (control), AZT 1 μ M and AZT 10 μ M.

Grupo	Number	Median	Lower Quartile	Upper Quartile	P value
MII oocyte	28	2.14 ^{a,b,c}	0.63	2.74	< 0,001
Control	21	3.13 ^{a, d}	2.61	3.75	
AZT 1 μ M	21	3.55 ^{b, d, e}	3.2	4.3	
AZT 10 μ M	24	2.84 ^{c, e}	2.49	3.52	

Note. P value according to Kruskal Wallis' test. Similar letters in the same column indicate a significant difference ($p < 0.05$) by Dunn's posttest. ^a: $p = 0.002$, ^b: $p = 0.001$, ^c: $p = 0.001$, ^d: $p = 0.02$, ^e: $p = 0.004$.