Journal of Assisted Reproduction and Genetics (2021) 38:3145–3153
https://doi.org/10.1007/s10815-021-02331-w

EMBRYO BIOLOGY

Inhibition of LINE-1 retrotransposition represses telomere reprogramming during mouse 2-cell embryo development

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Received: 14 June 2021 / Accepted: 23 September 2021 / Published online: 7 October 2021

Abstract

Purpose To investigate whether inhibition of LINE-1 affects telomere reprogramming during 2-cell embryo development.

Methods Mouse zygotes were cultured with or without 1 μM azidothymidine (AZT) for up to 15 h (early 2-cell, G1/S) or 24 h (late 2-cell, S/G2). Gene expression and DNA copy number were determined by RT-qPCR and qPCR respectively. Immunostaining and telomeric PNA-FISH were performed for co-localization between telomeres and *ZSCAN4* or LINE-1-Orf1p.

Results LINE-1 copy number was remarkably reduced in later 2-cell embryos by exposure to 1 μM AZT, and telomere lengths in late 2-cell embryos with AZT were significantly shorter compared to control embryos ($P = 0.0002$). Additionally, in the absence of LINE-1 inhibition, *Dux*, *Zscan4*, and LINE-1 were highly transcribed in early 2-cell embryos, as compared to late 2-cell embryos ($P < 0.0001$), suggesting that these 2-cell genes are activated at the early 2-cell stage. However, in early 2-cell embryos with AZT treatment, mRNA levels of *Dux*, *Zscan4*, and LINE-1 were significantly decreased. Furthermore, both *Zscan4* and LINE-1 encoded proteins localized to telomere regions in 2-cell embryos, but this co-localization was dramatically reduced after AZT treatment ($P < 0.001$).

Conclusions Upon inhibition of LINE-1 retrotransposition in mouse 2-cell embryos, *Dux*, *Zscan4*, and LINE-1 were significantly downregulated, and telomere elongation was blocked. *ZSCAN4* foci and their co-localization with telomeres were also significantly decreased, indicating that *ZSCAN4* is an essential component of the telomere reprogramming that occurs in mice at the 2-cell stage. Our findings also suggest that LINE-1 may directly contribute to telomere reprogramming in addition to regulating gene expression.

Keywords Mouse embryo · Telomere · Telomere length · Retrotransposition · Long Interspersed Nuclear Element-1 (LINE-1) · *Zscan4* · *Dux*

Introduction

Transposable elements (TEs) make up approximately 50% of the human genome [1]. They are sequences of, what were originally, viral DNA capable of self-replicating and inserting themselves into the host genome. In somatic cells, they are transcriptionally inactive and once were thought to be "junk" DNA, relics of ancient evolutionary processes [2]. Indeed, activation of TEs in somatic cells has been linked to some pathologies, including cancers and autoimmune diseases [3]. More recently, however, a few TEs were discovered to play crucial roles during normal early embryologic development [4]. There are two broad categories of TEs [5]. Class I TEs are known as retrotransposons, and common examples are endogenous retroviruses (ERVs) and LINEs. They require the formation of an RNA template from the original transposon DNA strand, which is then reverse-transcribed back to DNA to be re-inserted into the genome [6]. Class II TEs are DNA transposons. They do not require an RNA intermediate, but rather are able to jump to various parts of the genome [7].

Telomeres are non-coding repetitive nucleotide sequences found at the ends of DNA strands [8]. As a result of their unique sequences, they fold to form caps at the DNA tails, thereby preventing any attrition of the actual DNA strand

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with replication and protecting the DNA double strands from being exposed to the nuclear milieu [9]. Decreased telomere length has been associated with increased maternal age, and linked to infertility, and abnormal oocyte and embryo functioning [10–15]. From the zygote to the 2-cell stage, telomeres undergo rapid elongation at a time when telomerase is inactive, and at a rate beyond the capacity of telomerase [16, 17]. Telomere elongation is an essential step in the embryo's development. Because this elongation does not depend on telomerase, it is classified as an alternative lengthening of telomeres (ALT). ALT employs several mechanisms that use telomere DNA itself as a template [18, 19]. We previously have shown evidence of ALT during early mouse development [16], but it is still unclear which factor mediates.

During early embryo development, zygotic DNA is de-methylated to erase previous parental influence and achieve totipotency [20, 21]. This results in activation of retrotransposons that were previously kept transcriptionally silent by methylation [22]. Zygotic genome activation (ZGA) takes place when the 2-cell embryo transitions from relying on maternal RNA transcripts to producing its own mRNA [23–25]. For instance, LINE-1 is activated during ZGA, and inhibition of LINE-1 results in preimplantation embryo developmental failure [4]. LINE-1 RNA recruits Nucleolin/Kap1 in mice to repress *Dux*, another ZGA transcriptor highly expressed during the 2-cell stage [26], and subsequently facilitates the progression from the 2-cell stage to the 4-cell stage [27]. A *Zscan4* (Zinc finger and SCAN domain containing 4) gene is found to be only transcript at the late 2-cell stage during mouse preimplantation embryo development, and reduction of *Zscan4* transcript level delays 2-cell embryos' progression that ultimately leads to implantation failure [28, 29]. Moreover, *Zscan4* is essential for mouse embryonic stem cells (ESCs) to maintain telomere length and long-term genomic stability [28, 29], as inhibition of *Zscan4* results in shortened telomeres, chromosomal abnormalities, and developmental arrest [29]. Because *Zscan4*, and therefore telomere elongation, is linked to expression of retrotransposons during ZGA in mouse 2-cell embryos, we postulated that inhibition of retrotransposition via a LINE-1 inhibitor, zidovudine (3′-Azido-3′-deoxythymidine; AZT), will interact with *Zscan4* and regulate telomere lengthening during the 2-cell stage of mouse embryos.

Materials and methods

Materials

Primers and DNA oligoes were synthesized by Integrated DNA Technologies (Table 1). Primer stock was reconstituted to 100 μM in TE buffer and later diluted with nuclease-free water, as required, for the PCR assay. DNA oligoes were dissolved in nuclease-free water according to the assay that was described in below section.

All reagents, unless specified, were purchased from Sigma and prepared according to their cited protocols. Anti-ZSCAN4C (Millipore, Cat# AB4340) and anti-ORF1p (Abcam, Cat# ab216324) were diluted to 1:100 in blocking solution (3% BSA/PBS containing 0.05% TritonX-100) prior to the first antibody incubation, and goat anti-rabbit DyLight 488 (Life Technologies, Cat# 35,552) was diluted with blocking solution to 1:500 prior to the secondary antibody incubation. The telomere probe (TelG-Cy3) was purchased from PNA Bio (Cat# F1006), made into 50-μM stock in formamide, and stored at − 20 °C.

Table 1 Sequence of all primers and DNA oligoes

Primer/DNA oligo	Sequence of forward primer	Sequence of reverse primer
Telomere	CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT	GGCTTGCCTTACCCTTACCCT TACCCTTACCCTTACCCT
LINE-1-Orf1p	GAACCAAGACCACTCACCATCA	CCCTGGACTGGGCGAAGT
B1	GCACCTTTAATCCCAGCAC	TGAGACAGGGTTTCTCTGTA
5S rDNA	GATCTCGGAAGCTAAGC	TACAGCACCCGGTATT
Zscan4d	CCGGAGAAAGCAGTGAGGTGGA	CGAAAATGCTAACAGTTGAT
Dux	CCCAGCGACTCAAACTCCTTC	GGACTTCGTCCAGCAGTTGAT
H2afz	AGGTAAAGCGTATCACCCCTC	CGATCAGCGATTTGTGGATGTG
LINE-1 mRNA	ATGGCGAAAGGCAAACGTAAG	ATTTTCGGTTGTGTTGGGGTG
ISPCR oligo	5-AAGCAGTGGTATCAACGCAGAGT-3	
Template-switching oligo	5-AAGCAGTGGTATCAACGCAGAGTACATrGrG + G-3	
Biotinylated Oligo-dT30VN	5-Biotin-TEG-AAGCAGTGGTATCAACGCAGAGTACTTTTTTTTTTTTTTTTTTTTTTTTTTTTTVN-3	

Mouse embryos in vitro culture

Mouse zygotes ($B_6C_3F_1$ X $B_6D_2F_1$) were purchased from Embryotech Laboratories and cryopreserved in liquid nitrogen. Mouse zygotes were thawed by following the product thawing instructions and then cultured in Global medium (LifeGlobal) containing 0.4% BSA, with or without 1 µM AZT, for up to 15 h (G1/S phase of early 2-cell stage), or 24 h (S/G2 phase of late 2-cell stage), in an atmosphere containing 5% CO_2 at 37 °C. At the time of collection, the 2-cell embryos were rinsed 3 times in 0.1% PBS/PVP buffer, and their zona pellucidas (ZP) were removed by short incubation in acidic Tyrode's solution. Denuded embryos were collected after washing 3 times in 0.1% PBS/PVP buffer, as required by the relevant assays.

Separation of gDNA and mRNA from mouse embryos simultaneously

Groups of fifteen denuded 2-cell embryos were collected into PCR tubes containing 2.5 µl of RLT Plus buffer (Qiagen) and either processed immediately or stored at − 80 °C. Genomic DNA and mRNA from embryos were extracted simultaneously by following the G&T-seq protocol with modifications [30]. First, we used 4 µl of Dynabeads per reaction to prepare oligo-dT30VN in order to separate gDNA and mRNA. Next, 25 µl of G&T-seq wash buffer was used to wash away the separated gDNA. A total of 38.5 µl of gDNA per reaction was separated and stored at − 80 °C for further analysis. Meanwhile 10 µl of reverse transcription (RT) master mix was prepared to generate cDNA from separated mRNA. Once the RT was finished, the cDNA product was immediately amplified by Kapa HiFi HotStart ReadyMix and IS PCR primer with total volume of 15 µl per PCR. Amplified cDNA was then stored at − 20 °C for further assessment.

Measurement of LINE-1 copy number and telomere length in mouse embryos by real-time PCR

Separated gDNA was first purified with 25 µl of Agencourt AMPure X beads, as described by the G&T-seq protocol. Once purified, it was then re-suspended together with the beads in 25 µl of pre-PCR master mix to amplify the targeted genes (Telomere, *B1*, LINE-1 and *5 s rDNA*). To prepare the pre-PCR master mix, we combined 0.25 µl of iTaq DNA polymerase (Bio-Rad), 0.5 µl of each primer pair (10 µM), 0.75 µl of $MgCl_2$ (50 mM), 0.5 µl dNTP mix (10 mM), 2.5 µl iTaq buffer (10×), and 19 µl of nuclease-free water together, per each reaction. After re-suspending the beads with pre-PCR master mix at room temperature for 5 min, all the samples were placed directly onto the thermocycler for pre-amplification. The program was set as follows: an initial denature at 95 °C for 3 min, followed by 15 cycles that included denaturing at 95 °C for 30 s, annealing at 60 °C for 30 s, and finally, extension at 72 °C for 30 s. After the gDNA was pre-amplified, it was purified with 25 µl of Agencourt AMPure X beads and eluted with 200 µl of nuclease-free water.

Gene copy number was measured with the SYBR green mix in a CFX96 Real-Time System. A total of 20 µl of PCR per well was setup by adding 10 µl of SYBR green mix (2×), 1 µl of primer pair (10 µM), 4 µl of nuclease-free water, and 5 µl of pure amplified DNA. Triplicate reactions per sample and per primer pair were set up in a 96-well plate and then run through program "CFX_2stepAMP" with anneal/extension temperature setting as 60 °C. The melting curve analysis showed the single peak of amplicon for each pair of primers, and relative gene copy number was calculated by the $2^{-\Delta\Delta Ct}$ method with 5 s rDNA as the internal control.

Relative quantification of gene expression in mouse embryos by real-time PCR

The amplified cDNA was purified with Agencourt AMPure X beads (Beckman) in a 1:1 volume ratio by following the G&T-seq protocol and later eluted with 50 µl of nuclease-free water. To measure the relative gene expression in mouse embryos, 5 µl of diluted cDNA (1:50 dilution with water) was added per reaction, as the DNA template, with an additional 15 µl of master mix containing 10 µl of SYBR green mix (2×) (Bio-Rad), 2 µl of primer pair (4 µM), and 3 µl of nuclease-free water. Triplicate reactions per primer pair were set for each sample, and then run through program "CFX_3stepAMP" with anneal temperature setting as 60 °C. The melting curve was run and confirmed the specific amplification for each pair of primers. A house keeper gene, *H2afz*, was chosen as the internal control, and the relative gene expression was compared to the control group (without AZT treatment) using the $2^{-\Delta\Delta Ct}$ method.

Immunofluorescence staining combining with telomeric PNA-FISH

Immunofluorescence staining followed by telomeric PNA-FISH was performed according to the cited protocol with modifications [31]. We placed denuded early 2-cell embryos individually on coverslips that were previously coated with 0.2% gelatin and dried for half an hour. The embryos were then left to rest for 20 min so they could firmly attach to the coverslip. Freshly prepared 3.7% paraformaldehyde (PFA) in PBS was used to fix the attached embryos in a 35-mm petri dish for 10 min at room temperature (RT). After washing with PBS 3 times, embryos were permeabilized with 0.2% TritonX-100 for 15 min and then blocked in blocking solution for 1 h at RT. The first antibody incubation was carried

in a humidity chamber overnight at 4 °C and was followed by the second antibody incubation in the same humidity chamber while avoiding light at RT for half an hour.

To perform telomeric PNA-FISH, embryos were fixed in 3.7% PFA for 10 min immediately after the secondary antibody incubation and washes, then washed three times in 2×SSC. Denaturation of embryos was performed with 50% formamide in 2×SSC for 30 min at 80 °C. Subsequently, denatured embryos were quenched in ice-cold 2×SSC buffer for 5 min. Embryos were dehydrated by step-wise passage through 70%, 85%, and 100% ethanol for 5 min each and air-dried for 15 min. Next, they were hybridized with 200-nM telomeric PNA probe in hybridization buffer. Incubation with the PNA probe was done in a humidity chamber at RT for 2 h, followed by 3 washes with 50% formamide in 2×SSC and 2 washes with 2×SSC buffer, in that sequence. Embryos with attached coverslips were then sealed on glass slides in Vectashield mounting medium with DAPI (Vector Laboratories). Images were taken under the Zeiss fluorescence microscope under 630× magnification and analyzed using ImageJ software with the object-based co-localization function by following the instruction online (https://imagej.net/imaging/colocalization-analysis).

Statistical analysis

Relative gene expression and copy number were presented as mean ± STD and analyzed with GraphPad Prism 8 software by one-way ANOVA Tukey's multiple comparisons test. Immunofluorescent foci were counted in groups of twenty nuclei and data was analyzed with GraphPad Prism 8 software by one-way ANOVA Mann–Whitney test. And $P < 0.05$ is accepted as statistical significance.

Results

Telomere elongation is restrained after inhibiting LINE-1 retrotransposition by AZT in mouse 2-cell embryos

AZT previously was shown to inhibit LINE-1 synthesis in mouse embryos and our data confirm this effect. LINE-1 copy number was significantly lower in late 2-cell embryos after AZT inhibition for 24 h, as compared to control embryos ($P < 0.0001$) (Fig. 1a). We also found that, among untreated embryos, LINE-1 DNA copy number was higher in late 2-cell embryos (1.941 ± 0.089) than early 2-cell embryos (1.334 ± 0.096) ($P = 0.0002$) (Fig. 1a), which indicates that LINE-1 retrotransposition occurs during S phase in 2-cell embryos. Additionally, as expected, telomeres elongated from early G1/S stage to late S/G2 stage (1.128 ± 0.059 vs 1.563 ± 0.107, $P = 0.001$) (Fig. 1b), which is consistent with our previous work showing that telomeres elongate during mouse 2-cell embryo development. Interestingly, exposure to AZT for 24 h blocked telomere elongation in 2-cell embryos, between early G1/S and late S/G2 (1.152 ± 0.039 vs 1.000 ± 0.107, $P = 0.199$). Moreover, telomeres in control embryos were significantly longer than in AZT-treated embryos (1.563 ± 0.107 vs 1.000 ± 0.107, $P = 0.0002$) (Fig. 1b). These findings indicate that inhibition of LINE-1 by AZT blocks telomere elongation during mouse 2-cell embryo development.

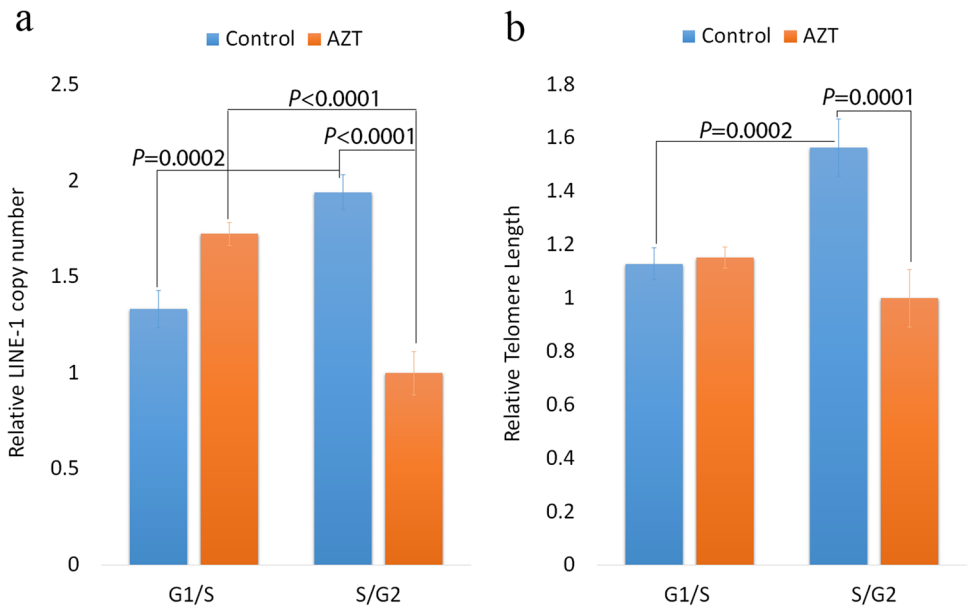

Fig. 1 Telomeres in mouse 2-cell embryos fail to elongate after inhibition of LINE-1 retrotransposition by AZT. (**a**) LINE-1 copy number in late 2-cell stage embryos after AZT treatment for 24 h is remarkably decreased when compared to control embryos by real-time PCR. (**b**) Relative telomere lengths in late 2-cell stage embryos are shorter than those of control embryos after AZT treatment for 24 h

Two-cell genes *Zscan4* and *Dux* are down-regulated along with LINE-1 transcripts in mouse 2-cell embryos with AZT treatment

Having shown that LINE-1 DNA synthesis is inhibited by AZT in mouse 2-cell embryos (Fig. 1a), we then asked whether LINE-1 and other 2-cell gene transcripts are also downregulated by AZT. As expected, AZT lowered LINE-1 transcripts in 2-cell embryos compared to the controls (1.266 ± 0.066 vs 1.910 ± 0.216, $P < 0.001$), suggesting that suppressing LINE-1 retrotransposition inhibits its transcription (Fig. 2). Notably, our data showed that transcripts of LINE-1, *Dux*, and *Zscan4* were more highly expressed in early 2-cell stage compared to late 2-cell stage ($P < 0.0001$) during normal 2-cell embryo development. This gives LINE-1 a similar expression pattern to other 2-cell stage genes, like *Dux* and *Zscan4*, which also are activated at the early 2-cell stage (G1/S). Strikingly, we observed that transcripts of *Dux* and *Zscan4* in early 2-cell embryos (G1/S) were significantly reduced by AZT treatment, in comparison with control embryos (0.312 ± 0.020 and 0.727 ± 0.054 vs 1.913 ± 0.197 and 1.913 ± 0.200, respectively, $P < 0.0001$). By contrast, expression of LINE-1, *Dux*, and *Zscan4* in late 2-cell embryos (S/G2) did not differ between AZT-treated and control embryos. Overall, our data indicates that inhibition of LINE-1 retrotransposition by AZT causes transcription suppression of LINE-1, *Dux*, and *Zscan4* during 2-cell embryo development.

ZSCAN4 locates on telomeres in 2-cell embryo nucleus and its co-localization with telomeres is reduced by AZT treatment

Next, we asked whether *ZSCAN4* interacts directly with telomeres in 2-cell embryos, since previous reports have shown that *ZSCAN4* is involved in telomere elongation by facilitating ALT in mouse ESCs. After immunostaining for *ZSCAN4* and LINE-1-ORF1p, as well as applying telomere PNA-FISH on fixed late 2-cell embryos, we were surprised to find that both *ZSCAN4* and LINE-1-ORF1p were specifically overlaid with telomeres in 2-cell embryo nuclei of the control group (Fig. 3a, b). When analyzing the effect of AZT on *ZSCAN4* and LINE-1-ORF1p level, we found a strikingly decreased count in the number of *ZSCAN4* foci and their overlay foci with telomeres in AZT-treated embryos compared to the controls, $P = 0.0002$ and $P = 0.0003$ respectively (Fig. 3b, e, f). This finding suggests that *ZSCAN4* plays the key role in telomere reprogramming during 2-cell embryo development. However, immunofluorescence staining shows only a slight decline in LINE-1-ORF1p foci between experimental and control embryos, $P > 0.05$ (Fig. 3a, c, d). Intriguingly, many LINE-1-ORF1p foci moved away from telomeres following AZT treatment (Fig. 3a), suggesting that LINE-1 plays multiple roles, including regulation of gene activation in addition to facilitation of telomere elongation.

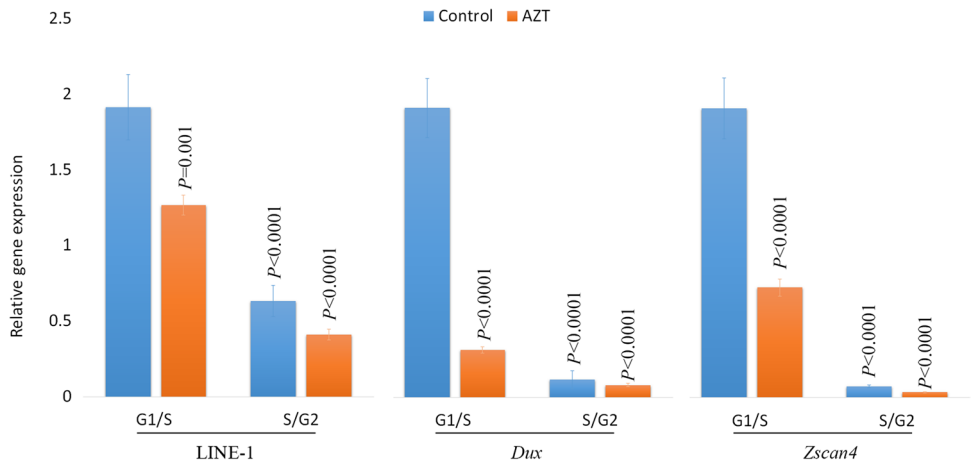

Fig. 2 Relative gene expression in mouse 2-cell embryos after AZT treatment for 15 h (G1/S phase) and 24 h (S/G2 phase) by RT-qPCR. LINE-1, *Dux*, and *Zscan4* were highly expressed in early 2-cell stage compared to late 2-cell stage without AZT exposure. And these genes were significantly downregulated in early 2-cell stage under AZT treatment compared to controls. In late 2-cell embryos, there were no significant differences when analyzing gene expression of LINE-1, *Dux*, and *Zscan4* between AZT-treated embryos and controls. All *P* values were calculated by comparing to control embryos at G1/S phase

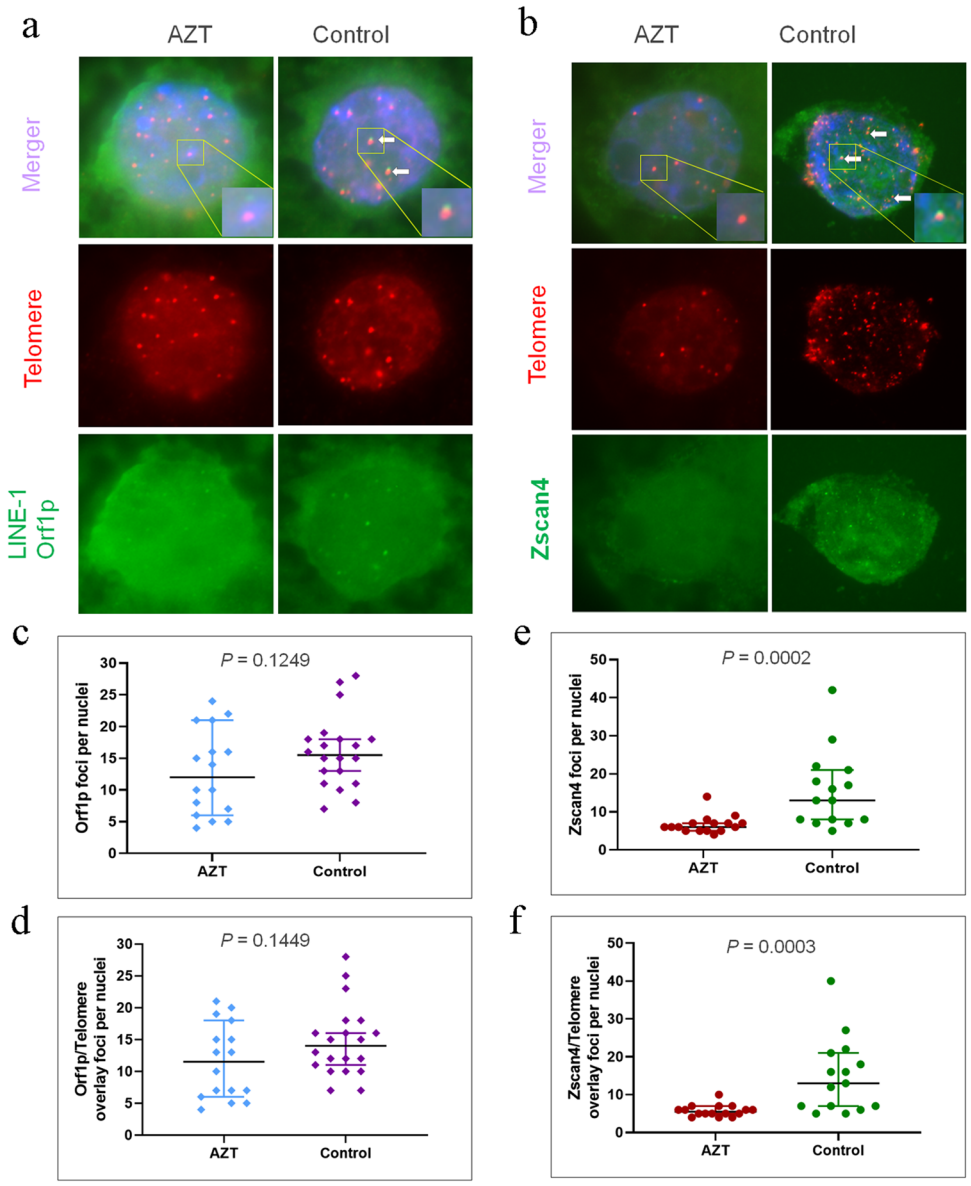

Fig. 3 LINE-1-Orf1p and *ZSCAN4* co-localization with telomeres in 2-cell embryo nucleus. (**a**), (**c**), (**d**) Immunostaining and PNA-FISH show LINE-1-Orf1p overlaid with telomeres in control embryos. Co-localization of LINE-1-Orf1p and telomeres was slightly reduced after AZT treatment. (**b**), (**e**), (**f**) Immunostaining and PNA-FISH show *ZSCAN4* foci in AZT-treated embryos were significantly decreased compared to control embryos, $P < 0.001$. Co-localization of *ZSCAN4* and telomeres was also dramatically reduced after AZT treatment, $P < 0.001$

Discussion

In vitro and in vivo experiments have shown that exposure to nucleoside analogue reverse transcriptase inhibitors (NRTIs), such as the HIV drugs AZT and lamivudine, arrests mouse preimplantation embryo development [32, 33]. This suggests that the administration of these drugs to women who are between ovulation and implantation could hinder embryo development by suppressing cell division [34]. NRTIs also inhibit LINE-1 reverse transcriptase activity [35, 36]. Since inhibition of LINE-1 activity by zygote microinjection of LINE-1 antibody or LINE-1 anti-sense oligonucleotides results in lower blastocyst embryo developmental rates [4, 37], it is possible that the effect of HIV drugs on embryo development is a consequence of inhibition of LINE-1 retrotransposition. LINE-1 is activated after fertilization and is essential during early mouse embryo development [27, 38, 39], but its precise role in 2-cell embryo development during major ZGA is still unknown.

AZT, the first NRTI for the therapy of HIV-1, is incorporated into DNA in place of thymidine, causing early termination of DNA synthesis and inhibiting reverse transcriptase activity [40]. Recently, it has been shown that NRTIs, including AZT, effectively block LINE-1 reverse transcriptase activity in vitro [35] and in cell-based retrotransposition assays [41]. Studies from Malki [42] and Jachowicz [38] have shown that AZT treatment at 1 μM successfully inhibits LINE-1 retrotransposition in mouse preimplantation embryos. Moreover, AZT treatment in cultured cells was shown to induce micronuclei, chromosomal aberrations, sister chromatid exchange, and other genotoxic effects [43]. Hence, in this study, we treated

mouse zygotes by adding 1 μM AZT into culture medium for 24 h to block LINE-1 reverse transcription at the 2-cell stage with minimum genotoxic effects. Our data confirms that LINE-1 was effectively inhibited by AZT and the inhibition of LINE-1 retrotransposition results in a failure of telomere reprogramming in mouse 2-cell embryos.

Human gametes possess particularly short telomeres and express little telomerase activity. After fertilization, zygotes undergo repeated cell divisions, multiplying from one cell to more than a hundred cells, and rapidly elongate their telomeres [44–46]. Telomere reprogramming during preimplantation development is critical for reproductive developmental competence [13, 14, 47]. Previous studies have shown that mouse embryo telomeres undergo remarkable elongation during the 2-cell stage, that this occurs even in the absence of telomerase, and that telomere sister chromatid exchange (T-SCEs) takes place, consistent with some form of alternative telomere elongation (ALT) [16]. Recently, *Zscan4* has received great interest as a gene that is expressed in only 5% of mouse ESC population, and its activation is involved in telomere elongation through ALT for telomere recovery accompanied rapid cell cycles in mESCs [48]. Because *Zscan4* is expressed at the 2-cell stage of mouse preimplantation embryos, coincident with rapid telomere elongation, we hypothesized that *Zscan4* plays a significant role in ALT during the 2-cell stage of development. Consistent with this, we found co-localization of *ZSCAN4* with telomeres in mouse 2-cell embryos and failure of telomere elongation following loss of *ZSCAN4*. Therefore, our findings provide direct evidence for the first time that *Zscan4* mediates telomere elongation in mouse 2-cell embryos.

To our surprise, LINE-1-ORF1p also co-localizes to telomeres in 2-cell embryos. Upon inhibition of LINE-1 retrotransposition, LINE-1-ORF1p leaves telomeres. No direct evidence demonstrates that LINE-1 mediates telomere elongation in mammals, but *Drosophila* maintain telomere length by transposition of specific non-long terminal repeat retrotransposons to chromosome ends [49, 50]. Due to the similarity between telomerase and LINE-1 reverse transcriptase, it is possible that LINE-1 plays a similar role in telomere reprogramming during the 2-cell stage of embryo development. Consistent with this model is the finding that human LINE-1 creates DNA double-strand breaks through its encoded endonuclease and increases the frequency of recombination [51, 52]. Our data also shows that LINE-1 localizes on telomeres in mouse 2-cell embryos, which indicates that LINE-1 may function as a facilitator of ALT by promoting DNA recombination and inducing DNA damage at telomeres. Therefore, we believe that telomere reprogramming in 2-cell embryos is mediated by *Zscan4* and LINE-1. How these two interact, however, requires further investigation.

During human early embryo development, telomeres also elongate after fertilization, as they do in mice [53]. Due to ethical concerns, it is difficult to obtain normal human embryos for research. Thanks to burgeoning single-cell technologies and next-generation sequencing, studying the molecular mechanisms in human early embryos is becoming more feasible. Since Zscan4 has been shown to be highly expressed in human 4-cell stage embryos, and further accumulates up to the 8-cell stage [54], we speculate that Zscan4 plays a role in telomere reprogramming at the 4–8-cell stage of development of human embryos, similar to that in mouse 2-cell embryos.

In conclusion, our findings report for the first time that *ZSCAN4* localizes to telomeres and mediates telomere elongation during mouse 2-cell embryo development. We further demonstrate that LINE-1 plays key role in this process, and that the functions of LINE-1 and *Zscan4* in telomere reprogramming during early development merit further study.

Funding This study was supported by March of Dimes Grant # 6-FY14-432 and the Stanley H. Kaplan Endowment.

Declarations

Conflict of interest The authors declare no competing interests.

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