ISSN 0026-8933, Molecular Biology, 2015, Vol. 49, No. 1, pp. 144–152. © Pleiades Publishing, Inc., 2015.
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CELL MOLECULAR BIOLOGY

UDC 577.21:575.224.23:618.32

Methylation Status of LINE-1 Retrotransposon in Chromosomal Mosaicism during Early Stages of Human Embryonic Development

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Received May 23, 2014; in final form, June 30, 2014

Abstract—Early stages of human embryonic development are characterized by the spatiotemporal coincidence of events of total epigenetic genome reprogramming and elevated level of mosaic forms of numerical chromosome abnormalities. It is possible that the abnormal reprogramming of various regions of the genome can lead to violations of local epigenetic chromatin organization and gene expression, which affect the correct chromosome segregation during mitosis. In this study, a comparative analysis of the methylation index of LINE-1 retrotransposon, which largely reflects the methylation profile of the genome, is performed in placental tissues of spontaneous abortions with complete and mosaic forms of aneuploidy and with a normal karyotype, as well as in the control group of induced abortions of the first trimester of pregnancy. It was shown that extraembryonic mesoderm and chorionic cytotrophoblast of spontaneous abortions with chromosomal mosaicism are characterized by the highest index of LINE-1 methylation among all studied groups. At the same time, the excessive hypomethylation of transposable genetic element was registered in spontaneous abortions with normal karyotype. We hypothesize that violations of parental genome demethylation during epigenetic reprogramming at preimplantation stages of development may be associated with an increased frequency of mitotic errors in chromosome segregation, which leads to the formation of a mosaic karyotype.

DOI: 10.1134/S0026893314060193

Keywords: LINE-1, aneuploidy, chromosomal mosaicism, epigenetic genome reprogramming

DNA methylation is an extremely dynamic process throughout the whole course of mammalian embryogenesis. The program of ontogenesis is triggered by the total epigenetic reprogramming of a zygote's genome intended to induce the pluripotency of blastomeres, after which tissue-specific profiles of gene expression are established. The paternal genome is hypermethylated in spermatozoids, while the genome of oocytes is less methylated. The differences in methylation between the gametes are particularly distinct at the level of methylation of interspersed repeats, such as LINE-1 [1]. At the preimplantation stage, paternal genome loses 5-methylcytosine located preferably in repeated sequences; then, passive demethylation of both parental genomes occurs, reaching methylation minimum at the stage of blastocyst. After implantation, DNA is remethylated, and the tissues-specific profile of genome methylation is established [1]. Errors in epigenetic reprogramming can lead to impairment of the individual development program and intrauterine embryo death.

It should be noted that, during the same period, at the preimplantation stage, the rate of chromosomal mosaicism sharply increases, which is a consequence of mitotic errors upon chromosome segregation. Approximately 80% of early human embryos obtained upon in vitro fertilization contain aneuploid blastomeres, despite that, under the same conditions, most zygotes (87.5%) possess a normal karyotype [2]. Notably, the rise of mosaicism coincides with the time of total demethylation of genome, which may lead to the temporal destabilization of chromatin. This spatiotemporal coincidence of cytogenetic and epigenetic processes raises the question on their interrelationship, two aspects of which can be considered theoretically. On one hand, chromosomal mutations that arise in somatic cells at the very first divisions can result from the errors of epigenetic reprogramming of genome, which also affect the genes responsible for controlling chromosome segregation during mitosis. On the other hand, chromosomal abnormalities of meiotic origin contained in a zygote can massively impair its epigenetic reprogramming upon the activation of the embryonic genome and lead to multiple changes in gene expression, which interferes with the normal course of ontogenesis.

Our previous data, which indicate that the rate of aberrant methylation of some cell cycle control genes in embryos with aneuploid karyotype is increased [3, 4], support the former mechanism. In 4.7% of

Abbreviations: EM, extraembryonic mesoderm; CT, cytotrophoblast.

embryos with chromosomal mosaicism, epimutations of genes *P14ARF* and *RB1* precede the rise of a mosaic karyotype [5]. The fact that a shift in the time of random inactivation of X chromosome and considerable differences in the profile of CpG methylation in gene promoters are observed in the extraembryonic tissues of spontaneous abortions of the first trimester of pregnancy with chromosome 16 trisomy, which is almost invariably the result of errors during meiosis I in the mother cell [6, 7], supports the latter mechanism. Similar processes proceed in placental tissues of fetuses with confined placental mosaicism of trisomy 16 [8]. However, it remains unclear whether the deviations, which results from an impairment of normal epigenetic reprogramming, involve individual genes or they mark some more substantial changes in the genome methylation profile, which are the reason for or the consequence of aneuploidy. This system-wide impairment of the epigenetic profile of the genome are reflected by the methylation index of the LINE-1 retrotransposon, which is frequently used as an index of the global level of genome methylation [9, 10]. Furthermore, the precision of LINE-1 methylation is a prerequisite for achieving its regulatory functions in embryonic development at both the stage of division [11] and differentiation of extraembryonic tissues [12–14].

The aim of the work was to study the correlation between the methylation index of LINE-1 retrotransposon and numerical chromosome abnormalities in extraembryonic tissues of spontaneous abortions of the first trimester of pregnancy with complete or mosaic aneuploidy.

MATERIALS AND METHODS

Subject of the study. Placental tissues of 51 spontaneous and 18 induced (control) abortions were subjects of the study. Spontaneous abortions were obtained from women with the clinical diagnosis of nondeveloping (or anembryonic) pregnancy established upon the results of dynamic ultrasound examination. Induced abortions were obtained from healthy women who did not wish to preserve the normally progressing pregnancy due to social reasons. The duration of intrauterine development of the embryos was 9.6 ± 2.5 weeks for spontaneous abortions and 9.0 ± 2.0 weeks for induced abortions, which is a statistically insignificant difference in the compared sets ($p = 0.24$). The data on gestation age determined from the results of an ultrasound examination were only available for some of the spontaneous abortions ($n = 29$, 7.4 ± 1.4 weeks). The difference between the gestational and fetal age reflecting the delay in the embryo development and the time from the moment of death to the moment of biological material collection was 2.8 ± 2.0 weeks for the group of spontaneous abortions.

Two types of tissue were analyzed, chorionic cytotrophoblast (CT) and extraembryonic mesoderm (EM), which originate from trophectoderm and epiblast of inner cell mass, respectively, and possess considerable differences in the pattern of genome methylation [15]. Morphologically different tissues were separated mechanically.

Cytogenetics analysis. Karyotype of spontaneous abortions in the culture of EM was established using the standard metaphase analysis. Additionally, FISH analysis using the DNA probes specific to centromeres of chromosomes involved in aneuploidy was performed to determine the rate of mosaicism and distribution of cells with aneuploid chromosomes over the tissues. To prepare the probes, bacterial clones containing plasmid insertions of centromere fragments of the chromosomes (kindly provided by M. Rocchi, Institute of Genetics, Bari, Italy) were used. The procedures for preparing a suspension of interphase nuclei from noncultured cells of extraembryonic tissues and perform an interphase FISH analysis were described previously [16].

Analysis of LINE-1 methylation. Genomic DNA was isolated from native (non-cultured) cells (upon mechanical separation of embryonic tissues) and treated with proteinase K during 16 h at 37°C followed by DNA purification with a mixture of phenol and chloroform. Bisulfite conversion of DNA was performed using the EZ DNA methylation Direct Kit (Zymo Research, United States) according to the manufacturer's protocol.

Methylation index of the LINE-1 retrotransposon was determined by pyrosequencing using the PyroMark Q24 CpG LINE-1 (Qiagen, Germany) kit according to the manufacturer's protocol. PCR samples contained a normal strength buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1 μL forward and reverse primer (10 pmol each), 2 units of HotStart Taq-polymerase (Qiagen), and 1 μL DNA (after bisulfite conversion) in the total volume of 25 μL. PCR conditions were as follows: primary denaturing, 15 min at 95°C; 45 cycles of 20 s at 95°C, 20 s at 50°C, and 20 s at 72°C; and terminal elongation, 5 min at 72°C. The size of the PCR product was 149 bp. Biotinylated PCR product was purified and the single-strand DNA was immobilized according to the manufacturer's recommendations. Then, 0.3 μmol of pyrosequencing primer were added to each sample and the reaction was run using the PyroMark Q24 pyrosequencer (Qiagen). Methylation index was calculated as the ratio of methylated cytosine to the sum of methylated and non-methylated cytosines using the PyroMark Q24 software at three CpG sites of the LINE-1 promoter region (Fig. 1).

Statistical analysis. LINE-1 methylation indexes were compared in the groups of embryos using the Mann–Whitney U-test. The correlation between the LINE-1 methylation index and the duration of prenatal development was evaluated using the nonparametric Spearman's test. To evaluate the prognostic value, ROC analysis was performed. Statistical procedures

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   1 gggggaggag ccaagatggc cgaataggaa cagctccggt ctacagctcc cagcgtgagc
  61 gacgcagaag acgggtgatt tctgcatttc catctgaggt accgggttca tctcactagg
 121 gagtgccaga cagtgggcgc aggccactgt gtgcgcgcac cgtgcgcgag ccgaagcagg
 181 gcgaggcatt gcctcacctg ggaagcgcaa ggggtcaggg agttcccttt ccgagtcaaa
 241 gaaaggggtg acggacgcac ctggaaaatc gggtcactcc cacccgaata ttgcgctttt
 301 cagaccggct taagaaacgg cgcaccacga gactatatcc cacacctggc tcagagggtc
 361 ctacgcccac ggaatctcgc tgattgctag cacagcagtc tgagatcaaa ctgcaaggcg
 421 gcaacgaggc tgggggaggg gcgcccgcca ttgcccaggc ttgcttaggt aaacaaagca
 481 gccgggaagc tcgaactggg tggagcccac cacagctcaa ggaggcctac ctgcctctgt
 541 aggctccacc tctgggggca gggcacagac aaacaaaaag acagcagtaa cctctgcaga
 601 cttaagtgtc cctgtctgac agctttgaag agagcagtgg ttctcccagc acgcagctgg
 661 agatctgaga acgggcagac tgcctcctca agtgggtccc tgacccctga cccccgagca
 721 gcctaactgg gaggcacccc ccagcagggc acactgacac ctcacacggc agggtattcc
 781 aacagacctg cagctgaggg tcctgtctgt tagaaggaaa actaacaacc agaaaggaca
 841 tctacacgaa aacccatctg tacatcacca tcatcaaaga ccaaaagtag ataaaaccac
 901 aaagatgggg aaaaaacaga acagaaaaac tggaaactct aaaacgcaga ggccctctcc
 961 tcctccaaag gaacgcagtt cctcaccagc aacagaacaa agctggatgg agaatgattt
1021 tgacgagctg agagaagaag gcttcagacg atcaaattac tctgagctac aggaggacat
1081 tcaaaccaaa ggcaaagaag ttgaaaactt tgaaaaaaat ttagaagaat gtataactag
1141 aataacc
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Fig. 1. Sequence of LINE-1 DNA retrotransposon (RefSeq X58075.1). The sequence under study in the promoter region is shown in the box; the CpG sites analyzed are marked in gray.

were performed using the Statistica 8.0 (Statsoft) software.

The study was approved by the Committee on Biomedical Ethics of the Research Institute of Medical Genetics, Siberian Branch, Russian Academy of Medical Sciences.

RESULTS

The standard cytogenetic analysis of 34 spontaneous abortions revealed trisomy of chromosomes 2, 7, 8, 9, 10, 13, 15, 16, 20, 21, and 22; the disomy of Ychromosome; and the monosomy of chromosome 13 among them. In all samples, percentage of the content of aneuploid clone was determined by FISH analysis while distinguishing between complete and mosaic forms of aneuploidy (Table 1). According to the data of FISH analysis, the fraction of aneuploid cells in abortions varied from 3 to 98% in CT and from 0 to 100% in EM. Taking into account the possibility of the overlap of signals of DNA probes specific to centromere in the course of FISH analysis, karyotype was considered mosaic if the fraction of aneuploid clone was less than 90%. As a result, subgroups of spontaneous abortions with complete and mosaic aneuploidy (27 and 7 embryos, respectively) were arranged (table); 17 abortions had normal karyotype.

Methylation indices of individual CpG sites in LINE-1 promoter did not differ significantly from one another in the abortion groups and correlated with each other well. Therefore, we used the average value of methylation indices for three sites further on. No differences in methylation indices between CT and EM were revealed in either group under study.

LINE-1 methylation indices did not differ in either CT or EM between spontaneous abortions with complete aneuploidy ($47.80 \pm 5.93\%$ and $48.11 \pm 4.67\%$, respectively) and induced abortions ($51.84 \pm 6.92\%$ and $52.72 \pm 3.75\%$, respectively) (Fig. 2). In the group of spontaneous abortions with mosaic aneuploidy, the LINE-1 methylation index was somewhat higher than in the group of induced abortions for both CT ($56.54 \pm 5.70\%$) and EM ($56.56 \pm 5.81\%$); however, these differences were only statistically significant in the case of CT ($p = 0.005$) (Fig. 2). Unexpectedly, LINE-1 methylation indices for CT and EM in the group of abortions with normal karyotype ($40.91 \pm 3.38\%$ and $43.38 \pm 6.17\%$, respectively) were also significantly lower than those in the group of spontaneous abortions ($p < 0.001$).

It should be noted that spontaneous abortions with pure and mosaic forms of numerical chromosome abnormalities have aneuploidies of different chromosomes; thus, the observed differences might have been the consequence of misbalance in the number of copies of different chromosomes in the compared subgroups. To exclude the influence of this factor, we compared LINE-1 methylation index in both extraembryonic tissues only in embryos with the most common aneuploidy of the first semester of pregnancy, that is, the trisomy of chromosome 16 (Fig. 3). Here, the LINE-1 methylation index was also significantly higher in CT of embryos with mosaic trisomy 16 ($56.27 \pm 5.71\%$) than in induced abortions ($51.84 \pm 6.92\%$) ($p = 0.012$). Furthermore, the significantly higher LINE-1 methylation index (if compared with induced abortions) is retained in the group of spontaneous abortions with the mosaic form of any other aneuploidy as well, with the exception of trisomy 16 ($p = 0.038$; data not shown). Therefore, the observed differences are not connected with the irregularities in aneuploidy distribution over different chromosomes in subgroups of embryos with mosaic and complete forms of numerical chromosomal abnormalities or with the fact that trisomy 16 is typical for spontaneous abortions.

Karyotype and the average LINE-1 methylation index (%) of three CpG sites in tissues of spontaneous abortions of the first trimester of pregnancy with aneuploidy of different chromosomes

No.	Cytotrophoblast		Extraembryonic mesoderm		Groups
	Karyotype	LINE-1	Karyotype	LINE-1	
1	47,XYY/46,XY (7:93)	NA	46,XY (100)	65.67	SA M
2	47,+16/46 (75:25)	NA	47,+16/46 (67:33)	65.33	SA M
3	NA	76.00	45,XX,−13/46,XX (19:81)	NA	SA M
4	47,XY,+16/46,XY (82:18)	71.00	47,XY,+16*	68.33	SA M
5	47,XX,+2/46,XX (87:13)	53.00	47,XX,+2*	54.67	SA M
6	47,XX,+15/46,XX (64:36)	54.33	47,XX,+15*	53.33	SA M
7	45,XX,−16/47,XX,+16/46,XX (10:8:82)	55.00	NA	49.67	SA M
8	47,XY,+22/46,XY (57:43)	65.67	47,XY,+22*	50.33	SA M
9	47,XX,+13/46,XX (58:42)	56.67	47,XX,+13/46,XX (67:33)	54.67	SA M
10	47,XY,+2/46,XY (32:68)	67.00	47,XY,+2*	66.33	SA M
11	47,XY,+8/46,XY (87:13)	56.67	47,XY,+8*	58.00	SA M
12	47,XX,+16/46,XX (40:60)	51.67	47,XX,+16/46,XX (83:17)	58.33	SA M
13	47,XY,+16/46,XY (67:33)	54.33	47,XY,+16/46,XY (82:18)	49.00	SA M
14	47,XX,+7/46,XX (99:1)	NA	47,XX,+7/46,XX [4:1]*	47.00	SA C
15	47,XX,+7/46,XX (98:2)	43.67	47,XX,+7 [4]*	NA	SA C
16	47,XX,+2/46,XX (98:2)	NA	47,XX,+2 [2]*	42.00	SA C
17	47,XX,+16/46,XX (52:48)	52.00	47,XX,+16/46,XX (65:35)	55.33	SA M
18	47,XX,+10/46,XX (18:82)	54.00	47,XX,+10/46,XX (30:70)	52.33	SA M
19	45,XY,−15/46,XY (18:82)	48.67	46,XY (100)	57.33	SA M
20	47,XX,+16/46,XX (98:2)	42.00	47,XX,+16/46,XX (98:2)	49.00	SA C
21	47,XX,+9/46,XX (72:28)	48.00	47,XX,+9/46,XX (60:40)	57.00	SA M
22	47,XY,+7/46,XY (3:97)	63.67	46,XY (100)	57.33	SA M
23	47,XX,+8/46,XX (3:97)	57.67	46,XX (100)	61.67	SA M
24	47,XYY/46,XY (68:32)	53.33	47,XYY (100)	57.00	SA M
25	47,+16/46 (87:13)	57.33	47,+16 [2]*	NA	SA M
26	47,XY,+21/46,XY (74:26)	50.67	47,XY,+21/46,XY (80:20)	49.33	SA M
27	47,XY,+16/46,XY (24:76)	57.00	47,XY,+16/46,XY (5:95)	NA	SA M
28	NA	56.67	47,XY,+16/46,XY (92:8)	54.67	SA C
29	47,XX,+20/46,XX (85:15)	52.00	47,XX,+20 [12]*	NA	SA M
30	47,XX,+16/46,XX (74:26)	57.67	47,XX,+16/46,XX (81:19)	55.67	SA M
31	47,XX,+16/46,XX (81:19)	51.00	47,XX,+16 [8]*	NA	SA M
32	47,XY,+20/46,XY (92:18)	50.67	47,XY,+20/46,XY (92:18)	51.67	SA M
33	NA	51.67	47,XY,+20/46,XY (80:20)	50.67	SA M
34	47,XX,+16/46,XX (92:8)	46.00	47,XX,+16 [7]*	44.33	SA C

Fractions of clones with different karyotypes derived from FISH analysis results are indicated in parenthesis. The number of metaphase cells used to determine the karyotype in the course of the standard cytogenetic analysis is indicated in brackets. * indicates karyotype was determined based only on the results of the standard cytogenetic analysis; NA is not analyzed; SA M is spontaneous abortions with mosaic aneuploidy; and SA C is spontaneous abortions with complete aneuploidy. LINE-1 methylation index was determined at three CpG sites.

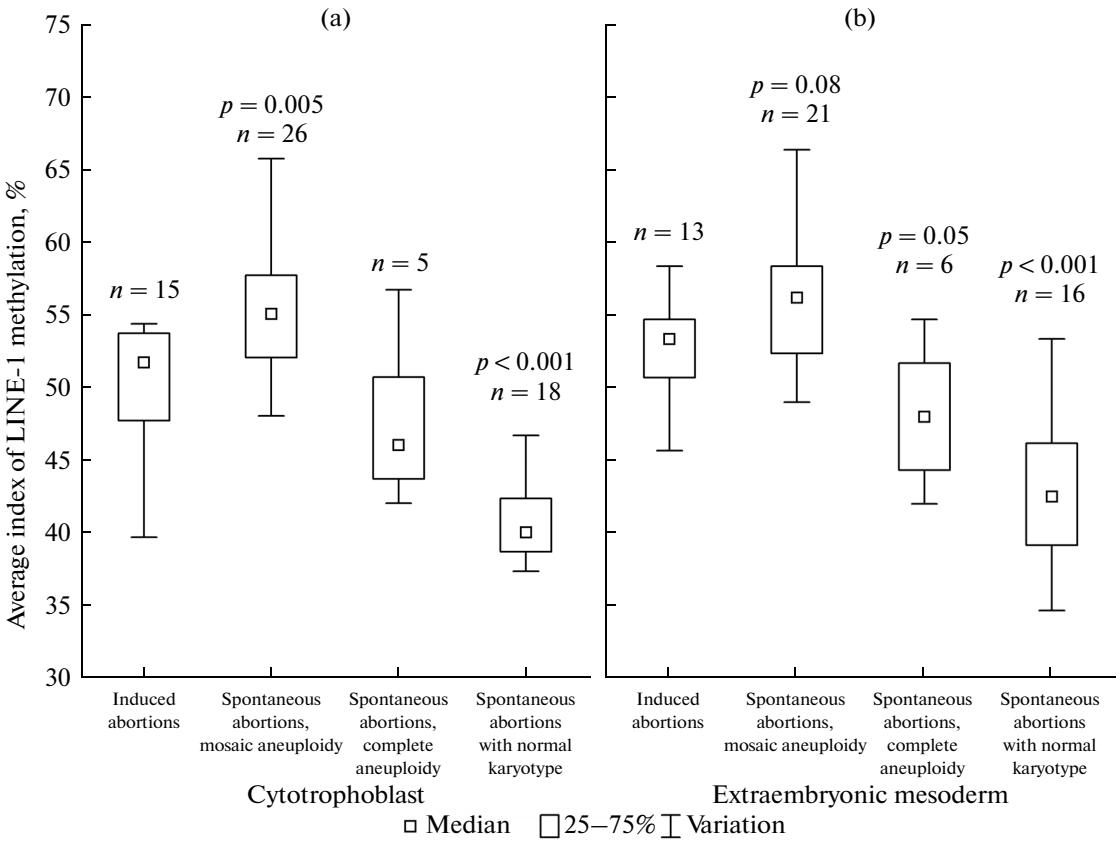


Fig. 2. LINE-1 methylation index in (a) chorionic cytotrophoblast and (b) extraembryonic mesoderm of induced abortions, spontaneous abortions with mosaic and complete aneuploidy of various chromosomes, and spontaneous abortions with normal karyotype.

In general, the duration of the intrauterine development of embryos could affect the DNA methylation profile of extraembryonic tissues at the stage of placenta formation. On the other hand, DNA methylation is a dynamic marker and, theoretically, may change after the development of the embryo has stopped, which reflects the adaptational capabilities of placental tissues, which maintain relatively autonomous development under conditions of nondeveloping or anembryonic pregnancy. Therefore, we studied whether there is a correlation between the LINE-1 methylation index and embryo age calculated from the date of the latest menstruation and the age determined from the ultrasound examination. It turned out that, in both cases, there were no significant effects. Furthermore, no correlation was observed between the LINE-1 methylation index and the difference between the embryo age calculated from the date of the latest menstruation and the age determined from the ultrasound examination. The latter parameter reflects the delay in the embryo development and the time from its death to the moment of biological sample collection. In other words, the retention of the embryo in the uterine cavity after the cessation of development produces no considerable effect on the registered level of genome methylation (at least, judging by the results of LINE-1 methylation index evaluation). This is typical of both spontaneous abortions with normal karyotypes and spontaneous abortions with complete and mosaic forms of aneuploidy.

ROC analysis of all spontaneous and induced abortions indicates that the average methylation index of three CpG dinucleotides in EM and CT has a high prognostic value for detection of embryos with mosaic aneuploid karyotype (area under the curve, AUC, is 0.81–0.88; Fig. 4). The most sensitive marker is the methylation index of the second CpG dinucleotide in LINE-1 promoter in CT, which allowed us to identify absolutely all embryos with the mosaic form of aneuploidy.

DISCUSSION

Errors in epigenetic reprogramming at the earliest stages of ontogenesis can rise in various regions of genome, including the repeated sequences. Among these sequences, LINE-1 mobile genetic elements form the most abundant autonomous family and make up approximately 17% of the human genome [17]. These elements can reproduce in the genome using the

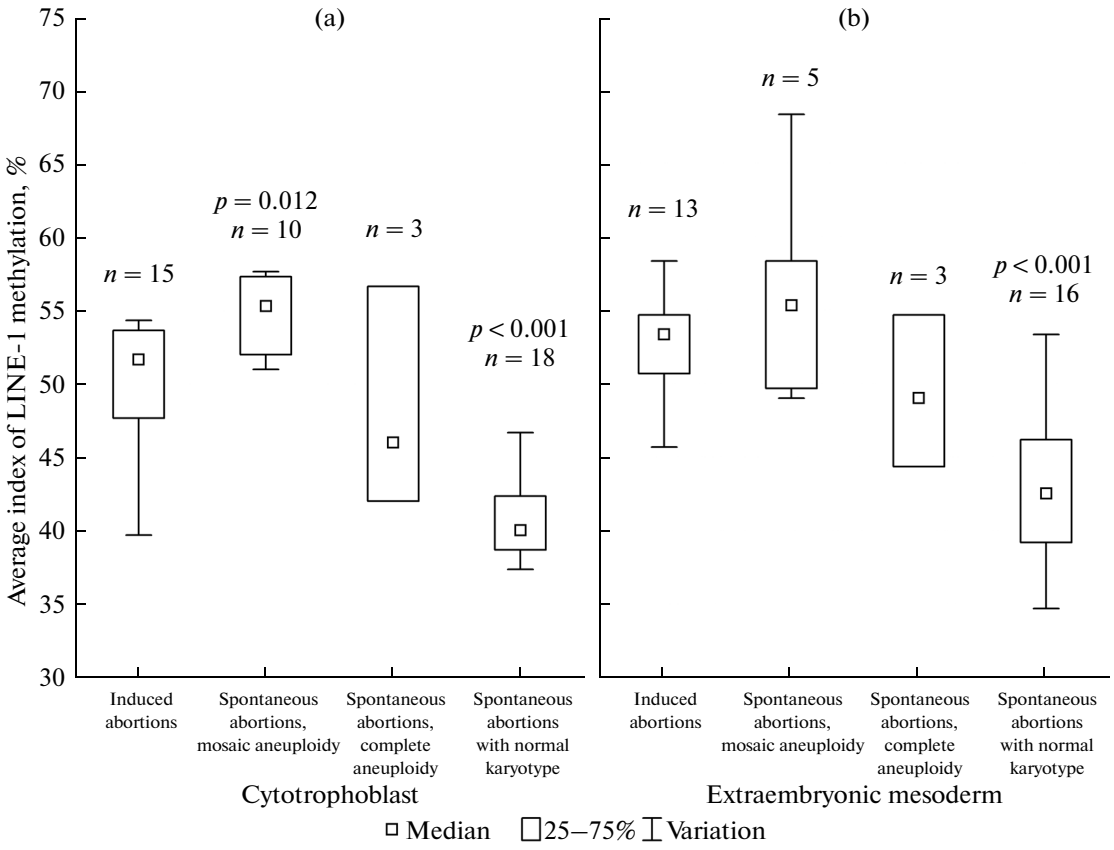
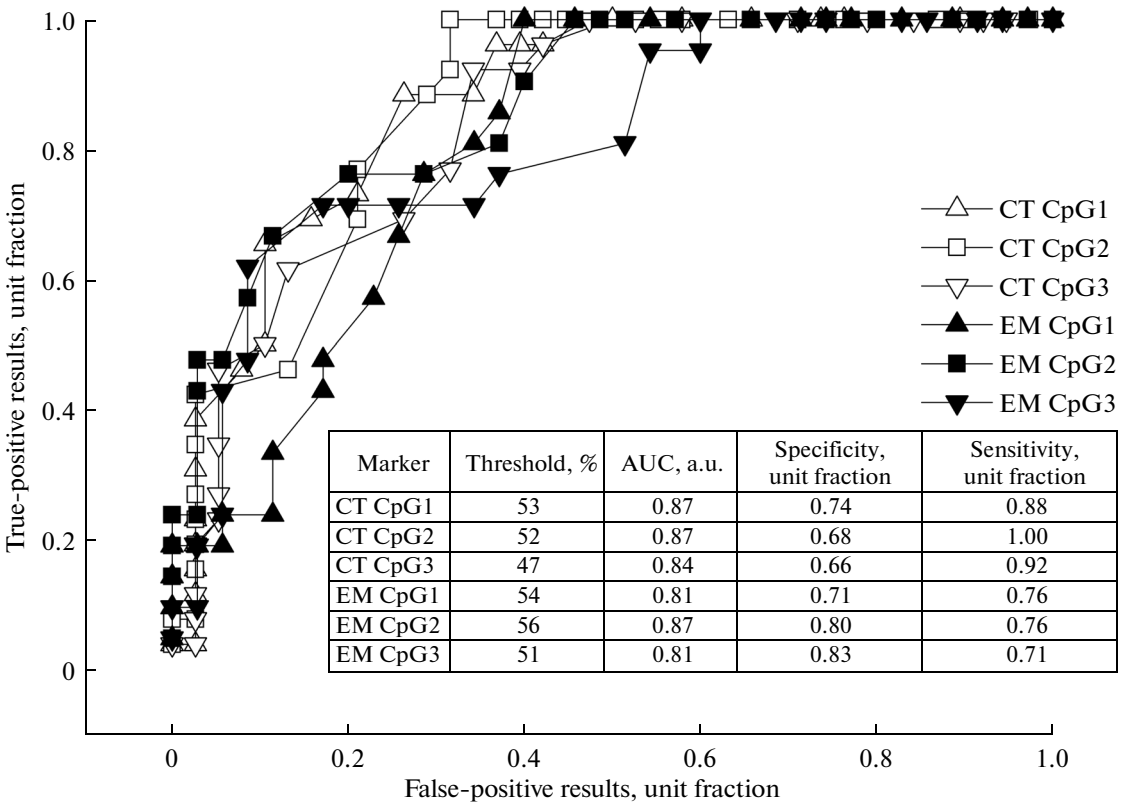


Fig. 3. LINE-1 methylation index in (a) chorionic cytotrophoblast and (b) extraembryonic mesoderm of induced abortions, spontaneous abortions with mosaic and complete trisomy 16, and spontaneous abortions with normal karyotype.

mechanism of reverse transcription; they also produce a considerable effect on the genome, mainly due to reverse insertion in new regions of the genome, for which expression of LINE-1 RNA and the encoded proteins is required. An increase in the level of LINE-1 elements' expression is accompanied by the partial demethylation of their promoters [18]. At the same time, activation of these mobile elements is necessary for the preimplantation stage of embryonic development. In particular, this is indicated by the fact that the promoter regions of the retrotransposon are partially demethylated at both the blastocyst stage and the extraembryonic tissues in mice and humans [19]. Microinjections of antisense oligonucleotides complementary to LINE-1 in murine blastocysts arrest retrotransposon transcription and lead to the irreversible abortion of the embryo development at the stage of two to four blastomeres [11]. Embryos remain viable, but stop dividing; the profile of gene expression changes considerably in these blastocysts. Furthermore, the suppression of LINE-1 expression leads to the impairment of chronological order in the activity of genes involved in the fulfilment of the ontogenesis program at the early stages of embryonic fission [11].

EM and CT of the chorion derive from different embryonic parts (epiblast, differentiated from the inner cellular mass, and trophectoderm, respectively). Earlier, we found that EM cells of embryos of the first trimester of pregnancy contain more methylated CpG sites located in gene promoters than CT cells [15]. However, contrary to this, LINE-1 methylation index in EM and CT cells in all studied groups of embryos, including the induced abortions, do not differ, as shown in the current work. This indicates that, apparently, starting from the cleavage, when cell mass and trophectoderm separate, the LINE-1 methylation index either does not change or these changes are of uniform and unidirectional nature in both extraembryonic tissues. Therefore, the data obtained in the current and previous studies evidence that the tissue-specific pattern of DNA methylation is determined mainly by the nature of methylation of gene promoter regions, and not the specific features of epigenetic modifications of the highly copied LINE-1 mobile genetic elements.

In the work, we demonstrate that, in placental tissues of spontaneous abortions of the first trimester of pregnancy with mosaic aneuploidy of various chromosomes, the LINE-1 methylation index is significantly elevated, while in groups of abortions with complete aneuploidy and induced abortions it takes the same values. Aneuploidy in embryonic cells can be of both



Marker	Threshold, %	AUC, a.u.	Specificity, unit fraction	Sensitivity, unit fraction
CT CpG1	53	0.87	0.74	0.88
CT CpG2	52	0.87	0.68	1.00
CT CpG3	47	0.84	0.66	0.92
EM CpG1	54	0.81	0.71	0.76
EM CpG2	56	0.87	0.80	0.76
EM CpG3	51	0.81	0.83	0.71

Fig. 4. Results of ROC analysis evaluating the possibility of using the LINE-1 methylation index in detection of embryos with mosaic aneuploidy. The figure shows the dependence of the number of true-positive results on the number of false-positive results upon using the threshold values of methylation index values for three CpG sites in LINE-1 promoter (CpG1–3) in cytotrophoblast (CT) and extraembryonic mesoderm (EM) to determine spontaneous abortions with mosaic aneuploidy. Sensitivity and specificity of individual markers, as well as the area under the curve, which indicates the prognostic value of a marker, are reported.

meiotic and mitotic origin, but independently of the origin of a genomic mutation, the rise of the mosaic variant of karyotype is always the result of mitotic errors that occur at the early stages of embryogenesis. Obviously, a gain or loss of a genes located in one of the chromosome in a zygote, or a chromosomal imbalance that arises at the early stages of cleavage can change the genome's epigenetic profile and lead to abnormalities in the dynamics of epigenetic reprogramming processes in early embryogenesis [20]. One of the waves of epigenetic reprogramming is known to occur at this stage of preimplantation development, when the methylation of parental pronuclei is removed. The paternal genome is subjected to active demethylation, which mainly involves repeated elements, including LINE-1, while passive demethylation occurs in the maternal genome concerning genes that encode proteins [1, 21].

The observed increase in the LINE-1 methylation index in spontaneous abortions of the first trimester of pregnancy with mosaic aneuploidy can be either the consequence or the reason for mosaicism appearance at the first stages of fission. In the former case, aneuploidy can lead to the impairment of the establishment of tissue-specific patter in DNA methylation, including the increase in LINE-1 methylation index. However, the hypothesis does not explain the absence of differences in LINE-1 methylation indices between different extraembryonic tissues of spontaneous abortions. On the other hand, the impairment of passive and active demethylation of parent genomes in a zygote, which is observed in some of embryos with normal or aneuploid karyotypes, can cause the rise of mosaicism in the course of the first cell divisions due to the lack of LINE-1 activity, which is required for the fission stage [11]. The fact that the maximum values of LINE-1 methylation index are typical of both of the studied extraembryonic tissues of spontaneous abortions with chromosomal mosaicism supports the latter hypothesis. Furthermore, errors in the course of demethylation in a zygote may lead to impairment in epigenetic regulation of genes responsible for chromosome segregation and cell cycle control. Indeed, previously, we have shown that, in extraembryonic tissues of embryos with the mosaic aneuploid karyotype, there are epimutations in certain cell-cycle-control genes, with an epimutation of the *RB1* gene being the most frequent [22].

Therefore, the registered increase in LINE-1 methylation index in spontaneous abortions of the first trimester of pregnancy with mosaic aneuploidy can probably be a consequence of the impairment of demethylation of parental genomes in a zygote, which leads to the suppression of LINE-1 activity. In turn, this can be connected with the rise of chromosomal mosaicism in the course of the first mitotic divisions. At the same time, in embryos with meiotic aneuploidy, which does not involve the impairment of genome demethylation in a zygote, apparently no rise of mosaicism occurs in the course of the first mitotic divisions, and the aneuploid karyotype is retained in all cells of the organism.

The most unexpected result of the current study is the fact that, in both extraembryonic tissues, in the group of spontaneous abortions with normal karyotype, the LINE-1 methylation index is equally decreased. This indicates that either LINE-1 methylation impairment originates from an early post-zygotic stage (prior to trophectoderm and inner cell mass segregation) or the absence of remethylation of the repeat in EM and CT of the chorion after implantation. The decrease in the level of LINE-1 methylation can lead to the increase of not only RNA expression, but the rate of transposition of the mobile genetic element, which increases the genome instability, which can be observed at other, noncytogenetic, levels of genome organization and probably leads to the intrauterine death of an embryo. Moreover, an increase in the number of LINE-1 copies can in turn cause an even stronger decrease in the methylation index.

Another possible explanation could be the impairment of the nutrient supply to the embryo, including the supply of folic acid, which serves as a major source of methyl groups. This impairment can lead to a global decrease in the level of the whole genome methylation, which we observe in the current work. Furthermore, the loss of DNA methylation theoretically could be the consequence of a response of extraembryonic tissues to the embryo death. However, we did not observe any statistically significant relationship between the LINE-1 methylation index and the period of time that has passed from the embryo death, which indicates that LINE-1 hypomethylation had started before the intrauterine development has stopped.

Thus, this is the first paper reporting that the low level of LINE-1 retrotransposon demethylation in a zygote can be associated with the rise of chromosomal mosaicism at the early stages of embryonic development. On the other hand, spontaneous abortions of the first trimester of pregnancy with normal karyotype also have abnormalities in the process of LINE-1 methylation, which is apparently accompanied by their intrauterine death.

ACKNOWLEDGMENTS

The work was supported by the Russian Foundation for Basic Research (project no. 14-04-01003).

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Translated by N. Kuznetsova