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Aberrations of the placental methylome and embryonic death

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Motivation and Aim: DNA methylation is one of the key mechanisms responsible for the implementation of the individual development program. The purpose of this study is to identify the potential role of multiple abnormalities in the methylome of extraembryonic tissues in the death of human embryos at early stages of development.

Methods and Algorithms: The methylation level was assessed using reduced representation bisulfite sequencing (RRBS) in chorionic villi of 8 spontaneous abortions of the first trimester of pregnancy with 45,X karyotype and 7 induced abortions with normal 46,XX and 46,XY karyotypes. Using STR analysis, the parental origin of the X chromosome was established in spontaneous abortions 45,X. Also, we analyzed 8 spontaneous abortions of the first trimester of pregnancy with normal karyotype with high and low level of LINE-1 methylation using RRBS.

Moreover, we analyzed the methylation profile of the LINE-1 retrotransposon using targeted bisulfite massive parallel sequencing in pairs of spontaneous abortions from the same families (n = 21 pairs).

Results: When spontaneous abortions with monosomy X of both maternal and paternal origin were compared to induced abortions with a normal karyotype of 46,XX and 46,XY, a number of differentially methylated CpG sites were found. Lamina-associated domains and subtelomeric regions of the genome were shown to have an enrichment of differentially methylation sites. A comparison with induced abortions with the 46,XX and 46,XY karyotypes revealed 831 and 254 differentially methylated genes (DMG), respectively. Genes of proteins with intrinsically disordered domains were enriched among genes with differentially methylation promoter regions in all spontaneous abortions with monosomy X. The two comparison groups shared 48 DMGs, of which 21 genes are important in normal placental and embryonic development and whose dysregulation is linked to preeclampsia and embryonic death. Furthermore, among the common DMGs with differences in the methylation index of ≥40 %, there are genes linked to the function of the Golgi apparatus and calcium channels in the cell membrane, which can cause pathologies in the excretory and cardiovascular systems in live births with Turner syndrome.

In spontaneous abortions with a normal karyotype, RRBS revealed large-scale anomalies in gene methylation, among which developmental protein genes were enriched.

Previously, we found an increased level of LINE-1 methylation in spontaneous abortions with an aneuploid karyotype. In this study, the level of methylation of the LINE-1 retrotransposon was found to be significantly correlated in spontaneous abortions from the same families (n = 21 pairs, R = 0.71, p = 0.0003). For pairs of spontaneous abortions with a normal karyotype (n = 10 pairs), the correlation remains significant (R = 0.74, p = 0.015), while for pairs of spontaneous abortions in which one of the abortions has

aneuploidy, the correlation becomes statistically insignificant (n = 8 pairs, R = 0.64, p = 0.08). If we exclude spontaneous abortions of half-siblings (n = 5 pairs), then the correlation also remains significant (n = 16 pairs, R = 0.67, p = 0.005). At the same time, no significant differences were found between the methylation level in all first abortions and all subsequent abortions in pairs (47.9 ± 6.9 % versus 43.9 ± 6.8 %, p = 0.062).
The role of the LINE-1 retrotransposon in the placenta is unclear. It was shown that among the genes capable of using LINE-1 promoters, genes expressed in the brain and placenta are enriched. In addition, retrotransposons from the L1PA2 subfamily may provide their antisense promoters as alternative promoters of long noncoding RNAs in the placenta. It is possible that LINE-1 elements incapable of retrotransposition gradually begin to be used by the genome as separate functional elements (primarily promoters) for nearby genes. In this case, an increase in the level of LINE-1 methylation can lead to disruption of the expression of nearby genes and disrupt the development of the placenta.
Conclusion: Aberrant methylation of genes involved in placentation, proliferation, and cell differentiation has been found in spontaneous miscarriages with monosomy X and normal karyotype. These disorders are likely to be the cause of the embryo lethality. The detected correlation between the levels of methylation of the LINE-1 retrotransposon in the chorionic villi of spontaneous abortions from the same families may be a consequence of common maternal factors affecting embryos and associated with their death, or indirect evidence of a transgenerational effect on the level of LINE-1 methylation of at least one from parents.
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