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Genome-wide abnormal DNA methylome in human blastocyst

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Abstract

Proper reprogramming of parental DNA methylomes is essential for mammalian embryonic development. However, it is unknown whether abnormal methylome reprogramming occurs and associates with the failure of embryonic development. Here we analyzed the DNA methylomes of 57 blastocysts and 29 trophectoderm samples with different morphological grades during assisted reproductive technology practices (ART). Our data reveals that the global methylation levels of high-quality blastocysts are similar (0.30±0.02), while the methylation levels of low-quality blastocysts are divergent and away from those of high-quality blastocysts. The proportion of blastocysts with a methylation level falling within the range of 0.30±0.02 in different grades correlates with the live birth rate for that grade. Moreover, abnormal methylated regions associate with the failure of embryonic development. Furthermore, we can use the methylation data of cells biopsied from trophectoderm to predict the blastocyst methylation level as well as to detect the aneuploidy of the blastocysts. Our data suggest DNA methylome may be a potential biomarker in blastocyst selection in ART.

Introduction

Epigenetic information plays critical roles during animal development[1-4]. The plasticity of epigenome enables cell differentiation, organogenesis, and animal development. On the other hand, proper epigenomic pattern at certain developmental stage is also required to ensure the totipotency of early embryos in animals[2]. Genome-wide DNA demethylation occurs during early embryogenesis in both human[5,6] and mouse[7,8]. The manually disturbing the DNA methylome reprogramming by genetic knock-out of DNA methyltransferases (DNMTs) or Tet3 in mouse results in a failure of embryonic development[9-11], indicating the importance of DNA methylome in mammalian development.

At present, approximately 12% of women of childbearing age in the United States have used assisted reproductive technology (ART) [12,13]. During natural pregnancy, the fertilized human eggs often abnormally develop, resulting in the miscarriage[14,15]. Likewise, during ART practice, significant proportions of human early embryos defectively develop and fail in producing the birth alive[16,17]. Genetic instability is speculated to be part of the reason, and indeed many kinds of mutations and chromosome errors are found in a proportion of these defective human embryos[18,19]. The frequency of aneuploidy in blastocysts significantly increased for women older than 35[20,21]. To avoid the genetic defects in early human embryos, preimplantation genetic diagnosis (PGD) has been widely used through the removal of a few cells from an embryo by biopsy and subsequent genetic analysis[18,19]. PGD for patients with good prognosis have resulted in the increased implantation and delivery rates[22,23]. Nevertheless, the majority of human embryos fail in producing birth alive for no known reasons. Although DNA methylation plays important roles during embryogenesis, it is unknown whether abnormal methylome reprogramming occurs during human embryonic development.

Material and Methods

Human samples

The human tissue collection and study procedure in this study were approved by the

Institutional Review Board at Peking University Third Hospital (Research license 2012SZ015). The methods closely followed the guidelines legislated and posted by the Ministry of Health of the People's Republic of China. The patients were informed of all details of the procedure, including sample utility and research destination. Patients voluntarily signed an informed consent document. Human embryos at the blastocyst stages were donated by the couples who had conceived at least one healthy baby by assisted reproductive technology (ART) treatment. These donor couples, whose infertility is purely due to female tubal factors, had a healthy baby through the IVF cycle already. They then donated the surplus frozen embryos for research with the written informed consents signed by them. Embryos were then graded according to Gardner morphological blastocyst grading system before collected for further methylation study.

The human embryos prepared for blastocyst biopsy were treated firstly at early blastocyst stage. One hole was made in the zona pellucida of these embryos opposite to ICM using laser pulse (ZILOS-tk, Hamilton Thorne Biosciences, Inc., Beverly, MA, USA). On day 6, the fully developed blastocysts were grouped and transferred into biopsy buffer medium (G-MOPES PLUS, Vitrolife). The ICM was fixed at the 9 o'clock position by the holding pipette, and the hached trophectoderm (TE) cells were stretched from the hole by a biopsy pipette with a 25 μm diameter. A few cells were removed from the blastocysts using laser pulse. The biopsied TE cells and the surplus blastocyst were then processed to methylome analysis.

Whole Genome Bisulfite Sequencing

Rather than mapping the methylome by pooling multiple blastocysts together, we investigated the methylome of each blastocyst individually. DNA Methylation libraries of human single blastocyst were constructed with our modified library generation method termed as "One Tube" method. Briefly, single blastocyst was lysed and then fragmented by sonication. The fragmented DNA was end-repaired, dA-tailed and ligated to cytosine methylated Illumina Truseq adapter. Bisulfite conversion reaction was performed directly on the ligation mix with 0.5% unmethylated lambda

DNA spiked-in. PCR amplified library was purified and sequenced on Hiseq2000 or Hiseq3000 platform (Illumina).

mRNA Sequencing

Blastocyst RNA was amplified and reverse-transcripted with REPLI-g WTA Single Cell Kit (Qiagen). RNA-seq Libraries were constructed with NEBNext® Ultra™ RNA Library Prep Kit for illumine (NEB) according to manufacture instruction. QC-passed libraries were then sequenced on Hiseq2500 platform with pair-end module. Methyl-seq and mRNA-seq data have been deposited in the GEO data repository under accession number GSE65736.

Sequencing Data Processing

Sequencing reads were trimmed to remove the reads containing adapters and low quality. Trimmed reads were aligned to human reference hg19 by using Bismark (v12.5) [24]. PCR duplications were removed with Picard (http://broadinstitute.github.io/picard/) and the overlapped regions in uniquely mapped paired reads were clipped with clipOverlap function of BamUtil (http://genome.sph.umich.edu/wiki/BamUtil: clipOverlap). CpG and non-CpG methylation level were extracted with mpileup function of Samtools (v0.1.19) [25]. Strands were merged to calculate the CpG methylation level per site. Average methylation level in each stage was the mean of methylation levels of each site. Principal Component Analysis (PCA) is often used to emphasize grouping structure in the data. We performed PCA analysis on the samples'%-methylation profiles by R package methylKit[26]. For PCA analysis, the methylomes with the genomic coverage higher than 15% were included. For DMR analyses in Figure 3, we used the R package bsseq, which is a smoothing local likelihood method that shows precise results even with low coverage data as well as have the ability to handle biological replicates[27]. DMRs contain at least 5 CpGs and the difference level between two groups higher than 0.2 were used for further analyses.

For mRNA-seq data, adapter-containing and low quality reads were trimmed and

then aligned with TopHat[28]. The unique reads were used to calculate the Fragments Per Kilobase of transcript per million mapped reads (FPKM) with Cufflinks v2.0.2 (http://cufflinks.cbcb.umd.edu). Differential gene expression between high- and low-quality blastocysts was calculated with DESeq using the default parameters [29].

The chromosome number was deducted by R package HMMcopy [30], which make copy number estimations based on read depth of the whole genome data in fixed interval with additional GC and mappability correction. The copy numbers were then segmented and classified with a robust Hidden Markov Model. Here we divided the genome into non-overlapping windows of 1Mb, and assigned the median autosomal read count corresponds to copy number 2. Chromosomal gain (copy number >2) and loss (copy number <2) were seen as horizontal green bars above and below, respectively, the copy number state of 2. Embryos were diagnosed as normal or euploid if the generated plot showed no gain or loss.

Results

Global abnormality of DNA methylome in human blastocyst

Since DNA methylome reprogramming is highly associated with embryonic development in animals[8,24], the precise reprogramming is, theoretically, important in determining embryonic condition. To investigate whether the abnormal methylome reprogramming occurs in human embryos, we analyzed the methylomes of blastocyst of various grades based on Gardner morphological blastocyst grading system including the ICM grade and the trophectoderm (TE) grade[25]. DNA methylomes at base-resolution were examined by using as low as single-cell (**Figure S1A**). We analyzed the methylomes of total 57 blastocysts with different morphological grades including high morphological grade (AA) blastocysts, middle grades (AB, BA, or BB) blastocysts and low grades (CC, BC or CB) blastocysts individually (**Figure S1B and Table S1**). Our data reveals that the global methylation levels of 12 AA blastocysts are similar (0.30±0.02, 95% CI 0.29-0.31), ranging from 0.27 to 0.32 (**Figure 1A** and **Table S1**). Surprisingly, the methylation levels of 12 (among 20) middle quality blastocysts do not fall within the range of 0.30±0.02 (**Figure 1A** and **Table S1**), but are variant. Furthermore, the methylation levels of 22 low quality blastocysts (among 25) differ from AA blastocysts (**Figure 1A**). The methylation levels of low-quality blastocysts are divergent, ranging from 0.23 to 0.46. Compared to high-quality blastocysts, low-quality blastocysts are more inconsistent in terms of methylation level (**Figure 1A**). These data demonstrate that the divergence of DNA methylome of blastocysts correlates with the morphological grades.

Additionally, the methylation state of functional elements in high-quality blastocysts is similar, while the methylation pattern of functional elements in each blastocyst of low-quality blastocysts displays a different state (**Figure 1B, Figure S1C and S1D**). Taken together, these data show that genome-wide abnormality of DNA methylome frequently occurs during human early embryonic development.

Abnormal methylated regions associate with pathways regulating embryonic development

To gain a close-up view of the differences observed above, we investigated differentially methylated regions (DMRs) between high-quality blastocysts and low-quality blastocysts. A significant proportion of DMRs are located in CpG islands (CGIs) or CGI shores (**Figure S2A and S2B**). The functional enrichment analyses demonstrated that the genes with promoters located in DMRs are enriched in many fundamental pathways critical for embryonic development, including cell cycle, DNA metabolism, chromosome localization and DNA modification (**Figure 2A**). Genes associated with intergenic DMRs are enriched in specific developmental categories, such as neuron development and spinal cord patterning (**Figure 2A**).

Interestingly, our data show that DNA methylation in enhancer regions are often falsely reprogramed in low-quality embryos. Gene ontology (GO) enrichment analyses for enhancers that locate within DMRs showed the enriched categories in developmental and metabolism pathways (**Figure S2C**). Figure 2B shows that enhancer for *IDH2* gene, a metabolism enzyme which can regulate the oxidization of 5mC[26], is hyper-methylated in CC blastocysts (**Figure 2B**). In consistence, the expression of IDH2 in three CC blastocysts is much lower than that in three AA blastocysts (**Figure 2C**). An example for a cell cycle gene, *CDK10*, is also differentially methylated in enhancers, and differentially expressed between high and low-quality blastocysts (**Figure S2D and S2E**). Furthermore, consistent with that divergence of DNA methylomes in low-quality blastocysts (**Figure 1B**), the global transcriptomes of CC blastocysts are also revealed divergent and different patterns from AA blastocysts (**Figure S2F**). Taken together, our results suggest that abnormal methylome may affect the developmental potential of early embryos.

Methylome status associates with the live birth rate following ART treatment

In ART treatments, AA blastocysts are ideal for embryonic transfer, which produce 39% live birth rate in Peking University Third Hospital. However, only a minority (33%) of the fresh elective single embryonic transfers (eSet) are AA blastocysts in our hospital, while the majority of the blastocysts for embryonic transfer (52%) are middle-quality B* blastocysts (AB, BA and BB) (**Figure S3A**). The live birth rate is

approximately up to 28% for either AB/BA blastocysts or BB blastocysts (**Figure S3B**). A small proportion of patients used the low-quality C* blastocysts (BC and CB), resulted in nearly 4% live birth rate (**Figure S3B**). Previously, the tested CC grade blastocysts were unable to produce any live birth[16,17], thus they were not used for embryonic transfer in our hospital. Considering that AA blastocysts have the highest live birth rate and uniform methylome, we regarded the methylome of AA blastocysts as good epigenomic status and used the average methylation level of AA blastocysts (0.30±0.02) as the control. Interestingly, the proportion of blastocysts with a methylation level falling within the range of 0.30±0.02 in different grades is correlated with the live birth rate for that grade (**Figure 3A**, Pearson Correlation Coefficient, r=0.93). The data suggest that DNA methylome status associates with the live birth rate in ART.

DNA methylome examination of cells biopsied from blastocyst

PGD has been used for more than 20 years to screen genetic diseases during ART practice. The removal of cells by biopsy from the trophectoderm has been used for the diagnosis of genetic mutations or chromosomal errors before embryonic transfer to the uterus[18,19]. Therefore, we use the cells removed by biopsy from TE in blastocyst to analyze the DNA methylome. The methylomes of 29 biopsied TE samples are profiled, 26 of them have paired methylome data of the blastocysts (**Table S1**). Our data show that the methylation levels of TE from high-quality and middle-quality blastocysts are similar to the paired blastocysts (**Figure S3C**). The methylation levels of most TEs from low-quality blastocysts are comparable to the levels of the paired blastocysts, except the levels of a few TEs are higher than the levels of the paired blastocysts, but the level are still significant different from control level (0.30±0.02) (**Table S1, Figure S3D**). Thus, our results show that DNA methylome of a few cells from TE in blastocyst with high and middle morphological grades can indicate the methylome of entire blastocyst. Notably, the P value of the homogeneity of variance between high-grade and low-grade TEs is 0.03, and the P value between high-grade and middle-grade is 0.05. These data indicate that that the methylation status of TE is

also correlated with the morphological grades of blastocysts (**Figure 3B**), which is similar to the results observed from blastocysts (**Figure 1A**). Therefore, as for the high-quality and middle-quality blastocysts, we are able to use the methylome of a few biopsied cells from TE to predict the DNA methylome of the blastocyst before the embryonic transfer to the uterus during ART. Since low-quality blastocysts will not be used in ART practices, it is unnecessary to examine its methylomes. Thus, hypothetically, the DNA methylome of a few TE cells removed from blastocysts could predict the methylome level of AA blastocysts (0.30).

Aneuploidy analyses from methylome data

Aneuploidy is frequently observed in human embryos. PGD has been widely used through the removal of a few cells from an embryo by biopsy and subsequent genetic analysis [18,19]. PGD for patients with good prognosis have resulted in the increased implantation and delivery rates[20-23]. PGD for Aneuploidy Screening (PGD-AS) has been applied in ART practices by array-based comparative genomic hybridization (arrayCGH) and single-nucleotide polymorphism (SNP) array approaches[27]. More recently, next-generation sequencing has been introduced into IVF field[28]. DNA methylation data have also been used to evaluate the copy number of chromosomes[29,30]. Therefore, we aimed to use our DNA methylome data to analyze the chromosome copy number variations (CNVs) in blastocysts and the biopsied TE cells. Notably, aneuploidy was present in 22 blastocysts (**Figure 3C, Table 1**), about 30% of all examined samples, which is consistent with the ratio discovered by traditional PGD [31]. Our data show that some blastocysts with normal methylation level still present with aneuploidy (**Figure 3D**), indicating that aneuploidy does not affect DNA methylation. Notably, the results in paired blastocyst and biopsied TE samples are highly consistent (**Table 1**), indicating that epigenomic examination on biopsied TE cells is predictive to the entire blastocyst. In summary, we can use DNA methylome from biopsied samples to predict the DNA methylome pattern and chromosome pattern, which may be useful for TE selection in the future.

Discussion

In this study, we performed whole genome bisulfite sequencing for 57 human preimplanted blastocysts as well as 29 biopsied TE samples individually. We found that global methylation level and pattern were dramatically unstable in low-quality embryos compared to high-quality embryos (**Figure 1**). We show that DNA methylome examination in biopsied TE can predict the entire blastocyst in both epigenetic status and chromosome aneuploidy.

DNA methylation reprogramming is highly associated with animal embryonic development[8,24]. The manually disturbing the DNA methylome reprogramming by genetic knock-out of DNA methyltransferases (DNMTs) or Tet3 in mouse results in a failure of embryonic development[9-11], indicating the importance of DNA methylome in mammalian development. The global methylome abnormality frequently takes place in human blastocyst, and associate with low live birth rate. Previous studies in human diseases including cancer show that the global methylation level in pathological tissue has limited (or no) change compared to normal tissue[32-34]. Considering that genetic manipulation of *DNMTs* in mouse can lead to the failure of embryonic development[9,10], the global abnormal methylome may be an important factor that results in the failure of human embryonic development. Indeed, falsely reprogrammed methylation regions are highly enriched in development related pathways (**Figure 2**), suggesting that the abnormal methylome may affect the developmental potential of embryos.

Right now, we are not sure what the mechanism behind the abnormal methylome is. Various pathways in different embryos may involve the causes of improper reprogramming. We have shown that aneuploidy does not associate with DNA methylation in blastocyst (**Figure 3D**). The advanced maternal age is associated with the rate of aneuploidy in blastocysts[20,21]. Probably, epigenetic status in blastocysts may also be affected by the maternal age. There is the likelihood that an improper micro-environment ultimately leads to an altered methylome in blastocysts since the environment has the ability to influence epigenetic states[35]. Frozen-thawed embryo transfer cycles may affect the stability of some proteins in early embryos, which could

also have the impact on the DNA methylome patterns. It is our hope that the optimization of embryo culture conditions during in vitro fertilization will facilitate methylation reprogramming and will improve the efficiency of ART treatment soon. In the future, more studies should be done to investigate the cause of the abnormality of DNA methylomes in human early embryos.

Right now, the outcome at birth is defined as a successful pregnancy. However, the consequences of ART practice for the baby's later life remains to be considered. Precious work has suggested that the intrauterine environment is associated with epigenetic programming of the fetal metabolism and predisposition to chronic metabolic disorders[36] later in life. Epimutation, especially these in imprinting disorders, is wildly observed after ART[37]. Therefore, a full understanding of the cellular and molecular biology of human reproduction must include a study of epigenetics and genomic imprinting. Furthermore, epigenetic variation during ART practice should be taken into consideration before embryo transferring.

PGD has been widely used to detect genetic mutations or chromosome errors before embryonic transfer to the uterus during ART clinic practices[18,19]. We show that DNA methylome examination can also detect aneuploidy in blastocysts. Our data further demonstrates that embryos with better methylome status associate with higher live birth rate (**Figure 3A**). Furthermore, our data suggest that epigenomic examination in blastocyst can determine the epigenomic status and euploid chromosome of the blastocyst prior to the embryonic transfer. Therefore, methylome examination in blastocyst may have advantage compared to traditional PGD method, and improve the efficiency of ART. Currently, 12% of women use ART services in the United States. In the future, proper clinic trial is needed to evaluate the value of DNA methylome examination method. We wish that the methylome examination in blastocyst might improve the efficiency of ART practices.

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Author Contributions

All authors contributed to the design of the study and critically revised the report for content. JQ and JL were responsible for the conception and overall supervision of the trial. YY and YF managed the samples gathering, with assistance from RL, XK, JY, YL, PL, YZ and HZ. GL and CL performed the experiments and data analyses, with assistant from XX, XM, JD and XP. XH, XS, and JL helped design the original protocol. YK and JQ provided technical advice and supervised the peer counseling intervention. GL, YY, and JL wrote the first draft of the report and were responsible for subsequent collation of inputs and redrafting. JQ and JL are guarantors for the report.

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Figures and legends

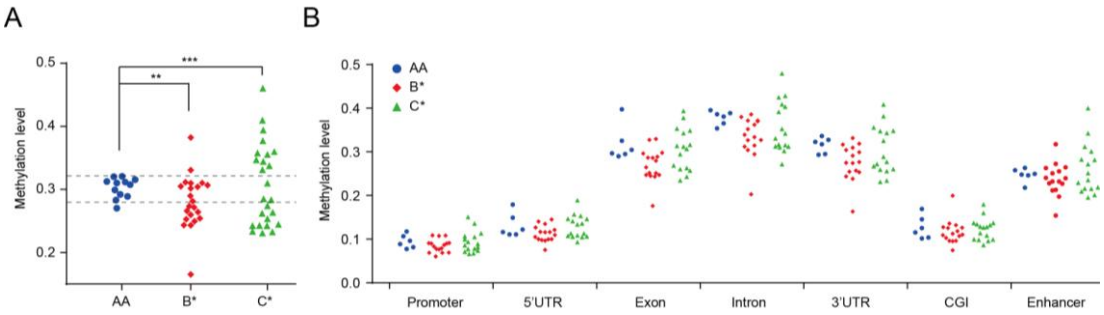

Figure 1. Global methylome instability in human early embryos.

(**A**) The methylation level of high-quality (AA), middle-quality (B*) and low-quality (C*) blastocysts. 1-4AA and 1-2CB blastocysts were derived from the same parents, as were the 1-6AA and 2-2CC blastocysts. The P value of the homogeneity of variance between AA and B* is 0.004 (**), P value between AA and C* is 7×10^{-5} (***).

(**B**) Average methylation levels of various functional elements in different blastocysts.

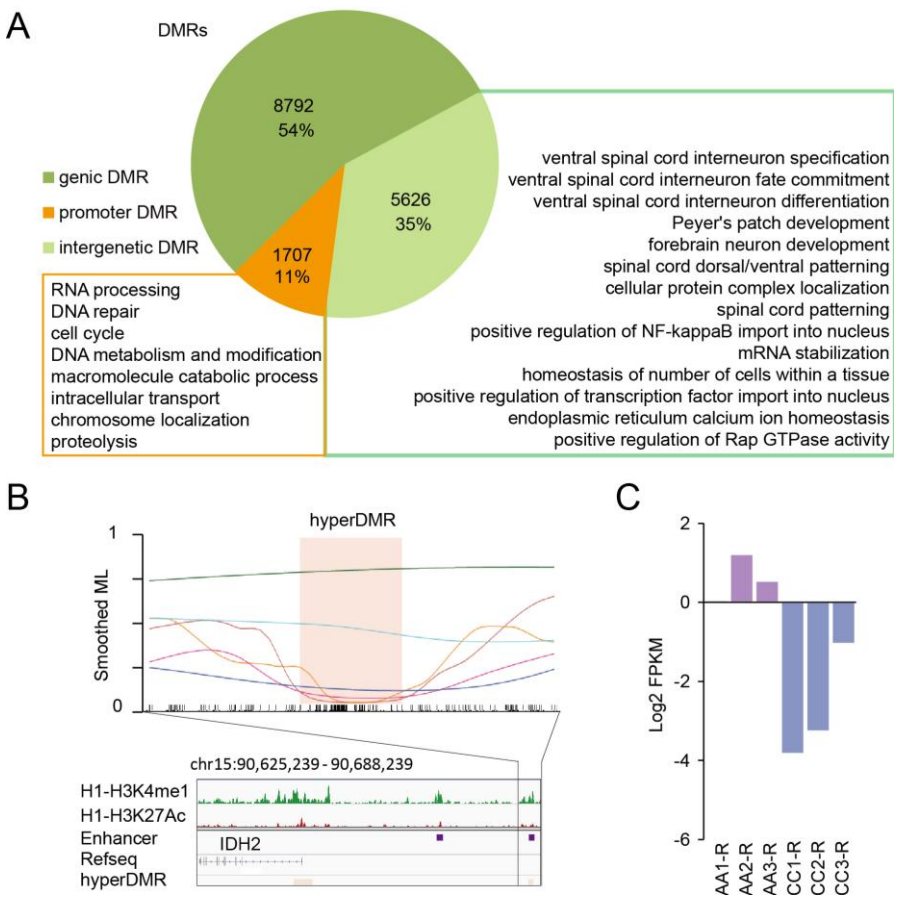

Figure 2. Functional analyses of altered methylation reprogramming.

(**A**) Functional enrichment of differentially methylated regions between high-quality and low-quality blastocysts. Genes with promoters located in DMRs were used for DAVID GO enrichment analyses. The cis-regulatory functions of intergenic DMRs were analyzed with GREAT tools. GO biological pathways with p-value < 0.05 were considered as the significant difference.

(**B**) The enhancer near IDH2 is differentially methylated. The smoothed methylation levels of high-quality and low-quality blastocysts are showed with extension ±5kb. The smoothed ML analysis was according to previous method [32]. Red, orange and purple indicate 1-4AA, 2-4AA and 1-6AA respectively. Blue, cyan, and blue violet indicate 9-3CC, 2-2CC and 8-3CC respectively. The DMR is indicated with pink shading. Short black bars indicate the location of CpG sites.

(**C**) The relative mRNA expression level of IDH2 in AA blastocysts and CC blastocysts. Three RNA-seq replications were performed for each group. P-value=0.03 (*).

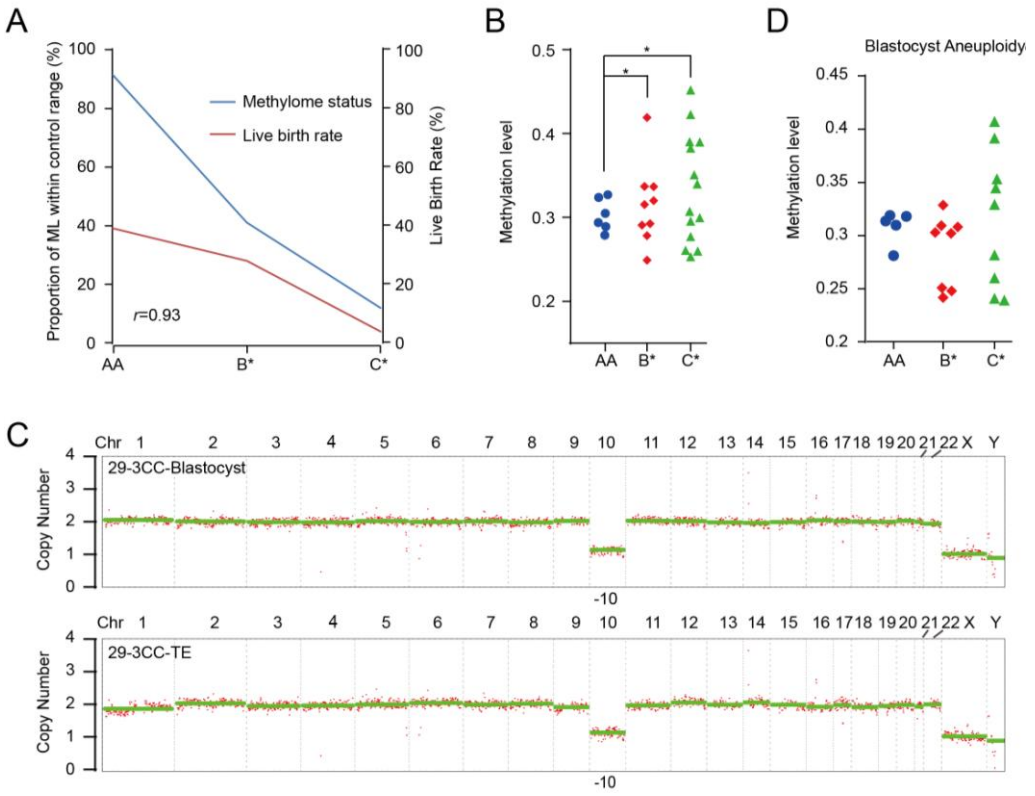

Figure 3. DNA methylome examination in human early embryos.

(**A**) The blue line indicates the proportion of blastocysts with methylation levels falling within 0.30±0.02. The red line indicates the live birth rate of the blastocysts with different grade. The result was from the patients using fresh embryos. The live birth rate of BC and CB is used to replace the live birth rate of BC、CB and CC blastocysts. The live birth rate of BC and CB should be higher than that of BC、CB and CC altogether.

(**B**) The methylation level of TE biopsied from high-quality (AA), middle-quality (B*) and low-quality (C*) blastocysts. * indicates p≤0.05.

(**C**) The graphical illustration of chromosome copy number was detected from our DNA methylome data.

(**D**) The methylation level of blastocysts with aneuploidy.

Table 1 Aneuploidy results for the embryos by using DNA methylome data.

	Sample	Aneuploid	Paired Sample	Aneuploid
AA	2-4AA	-19	6-5AA	-1p
	25-3AA	+5q, -9q	6-5AA-TE	-1p
			17-5AA	-22
			17-5AA-TE	-22
			19-4AA	-16
			19-4AA-TE	-16
B*	14-4BB	-13	11-5BB	-22
	54-5BB	-14	11-5BB-TE	-22
	32-6BA	+22	15-4BA	-20, -19
	13-5BB	-15, +17	15-4BA-TE	-20, -19
	31-5BB	-21, -22		
	18-5BB	-14		
C*	9-3CC	+7, X	29-3CC	-10
	2-2CC	-21	29-3CC-TE	-10
	36-3CC	+6	30-3CC	-12
	41-3CC	+16	30-3CC-TE	-12
			35-4CC	+16
			35-4CC-TE	+16
			50-4CC	XXY
			50-4CC-TE	XXY
			51-4CC	+4
			51-4CC-TE	+4

Supplemental Data

Supplement to: Guoqiang Li, Yang Yu, Yong Fan, et al. Globally abnormal DNA methylome in human blastocyst

Supplementary Figures 1-3
Supplementary Table 1

Supplementary Figures

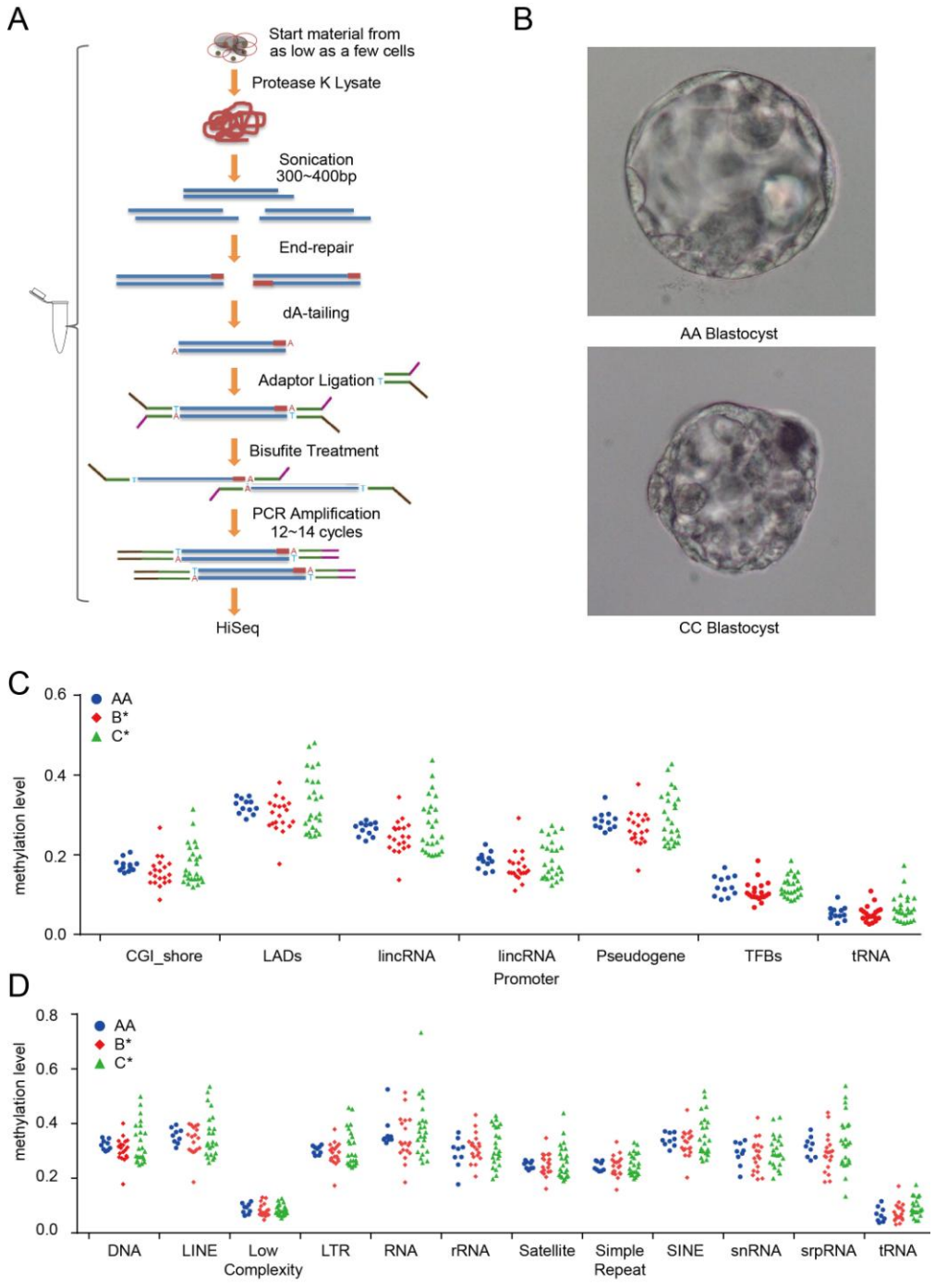

Figure S1. Epigenomic instability of blastocysts. (A) Illustration of the low-input MethylC-seq method for genome-wide methylome profiling. **(B)** Images of representative AA and CC blastocysts. (**C)** Average methylation levels of various functional elements in different blastocysts. (**D)** Average methylation levels of various repeat elements in different blastocysts.

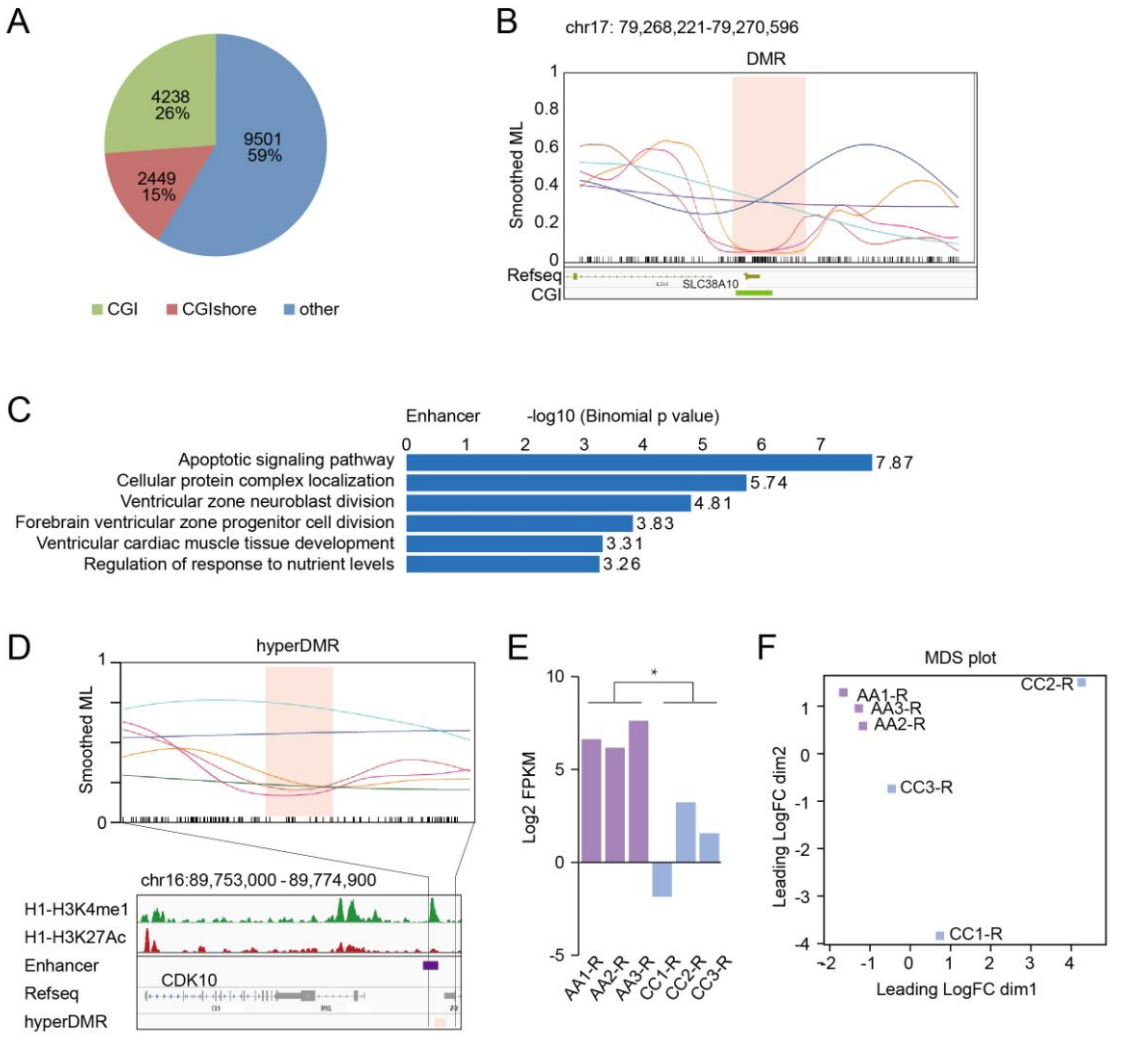

Figure S2. Functional analyses of altered methylation reprogramming. (**A)** The proportion of DMRs which locate in the regions of CGI or CGI shore. **(B)** Representative snapshot of a DMR (chromosome 17: 79,268,221-79,270,596) between high-quality and low-quality blastocysts. Smoothed methylation levels (sML) of high-quality and low-quality blastocysts are showed with extension ±5kb. Red, orange and purple indicate 1-4AA, 2-4AA and 1-6AA respectively. Blue, cyan, and blue violet indicate 9-3CC, 2-2CC and 8-3CC respectively. The DMR is indicated with pink shading. Short black bars indicate the location of CpG sites. (**C)** The GO enrichment of genes associated with differentially methylated enhancers. Terms with p-value < 0.05 were considered as significant enrichment. (**D)** The enhancer adjacent to gene CDK10 is differentially methylated. **(E)** The relative mRNA expression level of CDK10 in AA blastocysts and CC blastocysts. P-value = 0.05 (*). **(F)** PCA analyses according to the expression profile of each blastocyst.

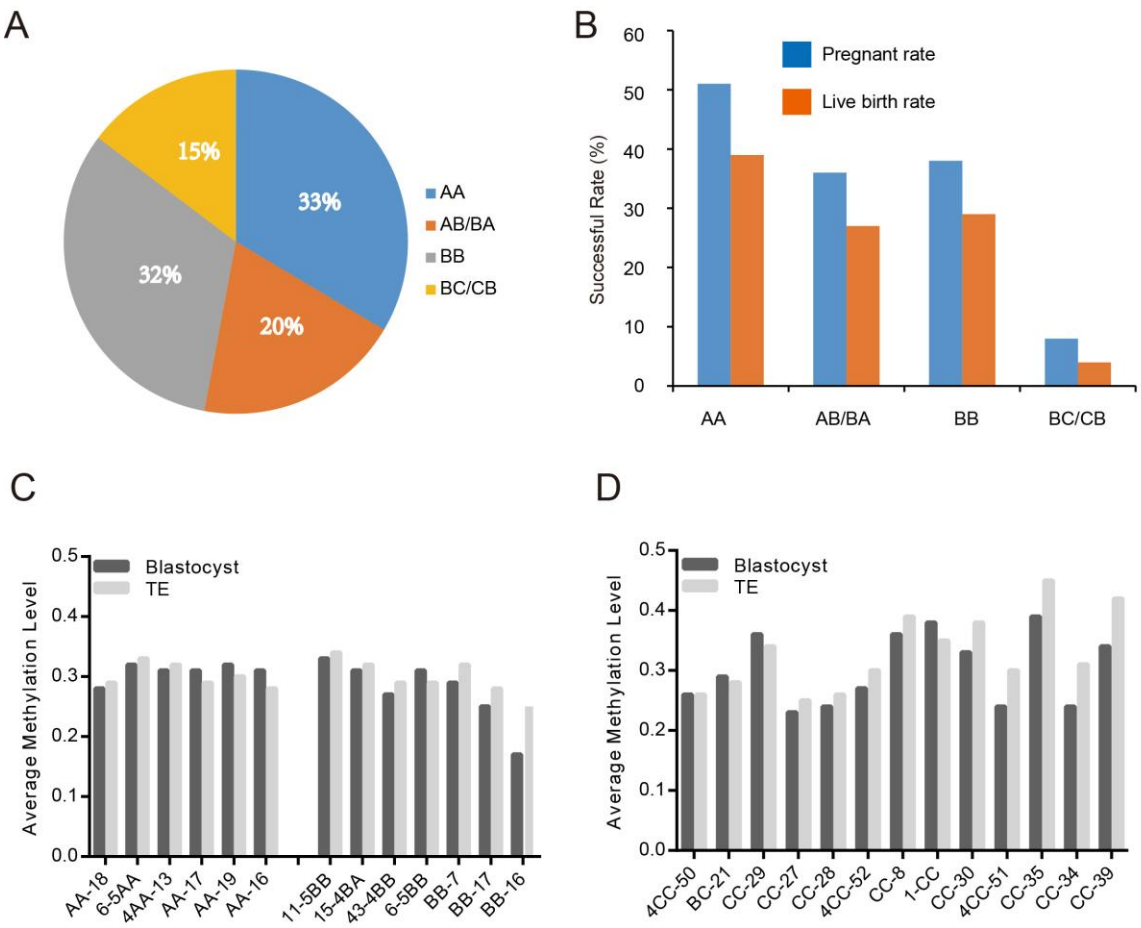

Figure S3. ART data for the blastocysts of different grades. (A) The proportion of different grade blastocysts used for fresh elective single embryo transfer (eSet) in ART practices. **(B)** The pregnant rate and live birth rate of the fresh blastocysts of different grades. **(C)** The methylation levels of the high-quality (AA) and middle-quality (B*) blastocysts with the paired TE samples. **(D)** The methylation levels of the low-quality (C*) blastocysts with the paired TE samples.

Supplementary Tables

Table S1. Summary of whole genome bisulfite sequencing of all blastocysts.

ID Blastocyst	ML	Cov (%)	BSCR (%)	ID Trophectoderm	ML	Cov (%)	BSCR (%)
1-4AA	0.30	71.92	99.01				
2-4AA	0.32	78.42	98.78				
1-6AA	0.27	67.08	98.81				
13-6AA	0.29	47.58	98.84				
14-5AA	0.31	48.02	98.58				
25-3AA	0.28	59.11	99.07				
6-5AA	0.32	13.72	98.62	**6-5AA-TE**	0.33	34.51	98.61
13-4AA	0.31	22.59	98.99	**13-4AA-TE**	0.32	33.07	98.99
16-5AA	0.31	12.17	99.14	**16-5AA-TE**	0.28	16.35	99.19
17-5AA	0.31	17.91	99.15	**17-5AA-TE**	0.29	29.68	98.14
18-4AA	0.28	17.97	98.96	**18-4AA-T**	0.29	24.65	99.10
19-4AA	0.32	33.45	98.92	**19-4AA-TE**	0.30	23.27	97.83
3-5BB	0.31	78.58	99.09				
4-6BB	0.28	72.80	99.05				
5-4BB	0.27	63.61	98.83				
6-4BB	0.38	79.96	98.72				
7-5BB	0.31	67.09	98.91				
8-4BA	0.26	71.19	98.83				
14-4BB	0.25	39.30	98.87				
11-6BB	0.27	29.34	98.56				
54-5BB	0.25	45.41	98.77				
32-6BA	0.24	33.60	98.73				
13-5BB	0.30	12.15	99.08				
31-5BB	0.30	17.94	99.13				
15-4BB	0.24	52.28	98.15				
6-5BB	0.31	11.12	99.13	**6-5BB-TE**	0.29	12.78	98.77
7-4BB	0.29	31.00	98.44	**7-4BB-TE**	0.32	6.00	98.64
16-5BB	0.17	62.96	98.82	**16-5BB-TE**	0.25	36.32	98.25
43-4BB	0.27	12.88	98.95	**43-4BB-TE**	0.29	9.69	98.91
17-5BB	0.25	33.73	98.59	**17-5BB-TE**	0.28	19.47	98.10

11-5BB	0.33	16.49	98.82	**11-5BB-TE**	0.34	16.48	98.67
15-4BA	0.31	16.60	98.30	**15-4BA-TE**	0.32	9.00	98.99
				13-4BB-TE	0.42	5.29	98.99
				18-5BB-TE	0.34	6.02	98.78
1-2CB	0.23	81.12	98.90				
10-4CC	0.23	53.41	98.85				
7-3CC	0.31	62.84	98.48				
9-3CC	0.35	36.39	98.97				
11-3CC	0.36	70.49	98.53				
2-2CC	0.41	47.21	99.00				
8-3CC	0.46	29.47	99.03				
13-CC	0.34	58.76	98.44				
36-3CC	0.28	50.06	98.94				
24-4CC	0.26	17.34	98.81				
33-4CC	0.25	57.73	98.97				
41-3CC	0.24	13.28	99.19				
14-4CC	0.36	10.00	98.88	**14-4CC-TE**	0.39	5.00	98.93
27-4CC	0.23	68.85	98.41	**27-4CC-TE**	0.25	18.32	99.18
28-4CC	0.24	13.10	98.83	**28-4CC-TE**	0.26	5.53	98.82
29-3CC	0.36	41.06	98.74	**29-3CC-TE**	0.34	43.29	98.82
12-4CC	0.38	6.92	98.25	**12-4CC-TE**	0.35	55.62	98.25
21-4BC	0.29	53.16	98.89	**21-4BC-TE**	0.28	41.90	99.08
30-3CC	0.33	54.54	98.81	**30-3CC-TE**	0.38	5.62	98.94
35-4CC	0.39	32.35	99.16	**35-4CC-TE**	0.45	4.84	98.93
39-3CC	0.34	54.10	98.87	**39-3CC-TE**	0.42	6.27	98.85
50-4CC	0.26	24.20	99.01	**50-4CC-TE**	0.26	35.89	98.26
51-4CC	0.24	46.52	98.56	**51-4CC-TE**	0.30	15.13	96.33
52-4CC	0.27	9.94	98.94	**52-4CC-TE**	0.30	17.56	99.01
34-4CC	0.24	38.49	99.14	**34-4CC-TE**	0.31	14.03	98.90
				38-3CC-TE	0.39	42.26	98.76

The quality of blastocysts was evaluated by Gardner blastocyst grading system, which includes the ICM grade and the trophectoderm grade. AA indicates both ICM and trophectoderm are good quality. CC indicates both ICM and trophectoderm are poor quality. CB indicates that the grade of ICM is poor and the grade of trophectoderm is middle. “ML” means average methylation level. “TE” means trophectoderm. “BSCR” means the bisulfite conversion rate calculated by spike-in unmethylated lambda DNA.

“Cov” means the proportion of mapped CpGs over total CpGs in human genome. There are 57 methylomes for blastocysts, and 29 methylomes of TE samples. 26 TEs have the paired blastocysts.