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LETTER TO THE EDITOR

Silencing of developmental genes by H3K27me3 and DNA methylation reflects the discrepant plasticity of embryonic and extraembryonic lineages

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Dear Editor,

One of the most important topics in mammalian embryogenesis is the generation of multiple cell lineages. Briefly, one single-cell totipotent zygote develops into the inner cell mass (ICM) and trophectoderm (TE) at the blastocyst stage. Afterwards, the ICM further generates the epiblast cells and finally forms multiple somatic cell lineages, while the TE develops into extraembryonic ectoderm (ExE) cells and eventually forms the placental tissue. The highly ordered programming of mammalian embryo development is spatial-temporally regulated by epigenetic mechanisms. Previous work has revealed that the ICM and TE remain largely epigenetically indistinguishable,[1, 2] even though there exist significant transcriptional distinctions. Recently we revealed the spatial-specific transcriptome of key development-related genes (DRGs) in mouse E7.0 gastrula, and we also noticed that these DRG-silenced gastrula regions possess distinct developmental potencies.[3] However, whether there are any distinctions of epigenetic mechanisms underlying the region-specific distribution of DRGs in post implantation embryos and how these epigenetic distinctions contribute to the regionalized developmental potential in mouse embryos remain unclear.

Here, we focused on two major epigenetic silencing mechanisms, H3K27me3 and DNA methylation (DNAme), in mouse post implantation embryos (Fig. 1a and Supplementary information, Figure S1A and B), which have been well characterized in pre-implantation embryos.[1, 2] The embryos were dissected into ExE and epiblast (Epi) at three consecutive stages, encompassing the last homogeneous epiblast stage (E6.5), the initial regionalized stage (E7.0) and the three germ-layer formation stage (E7.5)[4] (Fig. 1a and Supplementary information, Figure S1B). According to the regionalized developmental propensity, the E7.0 and E7.5 epiblasts were further dissected into anterior (A), posterior (P), and anterior mesoderm (AM, for E7.5 only) parts for high-fidelity epigenetic profiling, which can be indicated by region-specific marker expression (Supplementary information, Figure S1C and D). Given the limited cell number of early post implantation embryos, modified chromatin immunoprecipitation adapted for 10^4 cells[5] was utilized to profile the modification pattern of H3K27me3 and reduced representation bisulfite sequencing[6] was used to profile DNA methylome. Two biological replicates with high reproducibility (Supplementary information, Figure S1E, F and Table S1) were generated for each sample.

We observed weak dynamics of DNAme at post implantation stage from E6.5 to E7.5 stage (Fig. 1b). Noticeably, the ExE cells possessed lower DNAme level than the intraembryonic cells. Meanwhile, pervasive remodeling of H3K27me3 was identified during the development of the post implantation embryo (Fig. 1b and Supplementary information, Figure S2A). Clustering analysis revealed that significant distinctions of H3K27me3 and DNAme exist between pre and post implantation embryos (Supplementary information, Figure S2B and C). Interestingly, genomic distributions of H3K27me3 and DNAme exhibited negative correlation at multiple genomic regions, such as CpG islands (CGIs) and intracisternal A-particle (IAP) regions (Fig. 1b). Strikingly, most de novo H3K27me3 of post implantation embryos occurred at the regulatory genomic regions, such as CGIs. Moreover, weak dynamics of H3K27me3 can be identified at the repetitive regions, such as endogenous retrovirus K regions (ERVKs). Meanwhile, the increment of DNAme was observed at both the regulatory regions and repetitive elements. The different dynamics of H3K27me3 and DNAme suggested distinct roles of these two epigenetic modifications in mouse embryonic development. Differentially methylated region (DMR) analysis revealed that more unique methylation regions (4,862, change in methylation > 0.15, q-value < 0.05) can be identified in embryonic cells than in ExE cells (1,194; Supplementary information, Figure S2D). Surprisingly, most unique methylation regions in ExE were enriched for embryonic DRGs, such as neuron differentiation-related genes. In contrast, in the embryonic cells, specific lineage-related developmental genes were devoid of DNAme, and regions with higher DNAme were mostly surrounded by the genes associated with proteolysis and metabolic processes (Supplementary information, Figure S2E). Moreover, few DMRs were detected between intraembryonic cells (such as P and A; Supplementary information, Figure S2D). Given the generation of multiple lineages in the embryonic cells, alternative epigenetic silencing mechanisms must be employed to direct the diversification of embryonic lineages. It has been reported that H3K27me3 at high CpG content promoters (HCP) in the embryonic stem cells are vital for the regulation of developmental genes.[7] As to the mouse embryos, we found that the preference for different types of promoters was newly established in the post implantation embryos, and the preference of H3K27me3 for HCPs was much higher in embryonic cells of the post implantation embryos compared to the ExE (Fig. 1d; Supplementary information, Figure S2F and G). Given that the HCP-related genes are generally associated with ubiquitously expressed house-keeping genes and key developmental genes[8] and DRGs are uniquely methylated in the ExE cells, the higher preference of H3K27me3 for HCPs in embryonic cells may be the alternative epigenetic mechanism to direct the generation of multiple embryonic lineages.

By mapping H3K27me3 distribution and DNAme profiles around all 13,117 HCPs, we found that 13.4% (1,760/13,117) of HCPs exhibited differential distribution of H3K27me3 between ExE and embryonic cells (Fig. 1e and Supplementary information, Table S2). Further analysis revealed that among these 1,760 HCPs,

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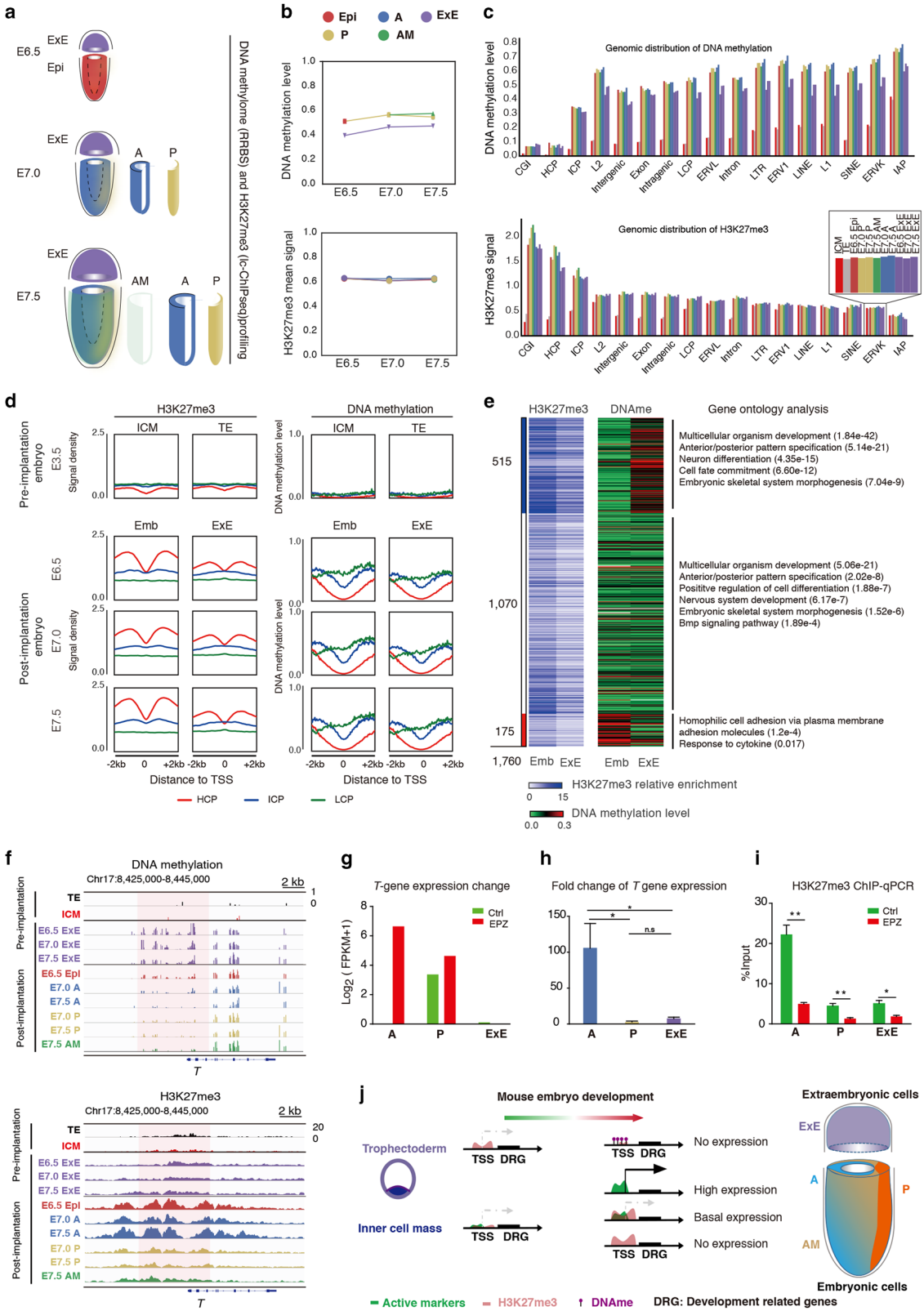

a large fraction of the genes (29.3%, 515 out of 1,760) had significantly higher DNAme in the extraembryonic cells than the embryonic cells ($P < 0.05$). Gene ontology analysis revealed that these 515 genes were mainly associated with embryonic patterning, neuron differentiation, cell fate commitment and other developmental processes (Fig. 1e). Previous study revealed that H3K27me3 usually represented bivalent or poised states of the regulatory elements, which facilitate the rapid response to proper developmental stimuli.[7] Thus, we hypothesize that this feature of H3K27me3 helps to poise the responsiveness of region-

Fig. 1 Distinct distributions of H3K27me3 and DNAme in mouse post implantation embryos. **a** An illustrative scheme for sample collection of the mouse gastrula. **b** Global DNA methylation (upper panel) and H3K27me3 dynamics (lower panel) during embryo development. **c** The genomic distributions of DNA methylation (upper panel) and H3K27me3 (lower panel). The published data of H3K27me3 enrichment[1] and DNA methylation[2] in pre-implantation embryos were used in these analyses. **d** Sequence preference of H3K27me3 and DNAme for different CpG content promoters at corresponding embryonic stages. **e** H3K27me3 enrichment and DNAme levels in the H3K27me3-dominant HCPs. These genes are further divided into three parts, top: DNAme(ExE)—DNAme(Emb) > 0.05; bottom: DNAme(ExE)—DNAme(Emb) < −0.05; middle: the rest of the genes. The result of gene ontology analysis was shown in the right panel. **f** The genome browser snapshot of H3K27me3 and DNAme at *T* locus in pre-implantation and post implantation embryos. **g**, **h** RNA-Seq (**g**) and RT-qPCR analysis (**h**) revealing the upregulation of *T* gene after EPZ treatment. $n = 5$. Student's *t*-test, $^{*}P < 0.05$, n.s. not significant. **i** ChIP-qPCR analysis revealing the dynamics of H3K27me3 enrichment at the promoter region of *T* gene with or without the treatment of EPZ. $n = 3$. Student's *t*-test, $^{*}P < 0.05$, $^{**}P < 0.01$. **j** A model describing that different epigenetic patterns at the loci of development-related genes facilitate mouse embryo development. RRBS, reduced representation bisulfite sequencing; lc-ChIPseq, low cell chromatin immunoprecipitation sequencing; HCP, high CpG content promoter; ICP, intermediate CpG content promoter; LCP, low CpG content promoter; ERVL, endogenous retrovirus L region; LTR, long terminal repeat element; ERV1, endogenous retroviral sequence 1; LINE, long interspersed nuclear element; SINE, short interspersed nuclear element

specific DRGs in their non-expressed embryonic regions and maintains the developmental competence of the early post implantation embryos, whereas unique DNAme enriched at key DRGs in ExE regions leads to a relatively permanent silencing status and restriction of extraembryonic lineages.

To test this hypothesis, we treated the dissected E7.0 embryo, which is the earliest stage for emergence of clear anterior-posterior asymmetry,[3] with a specific inhibitor of the PRC2 complex, EPZ005687 (EPZ),[9] to disrupt H3K27me3 in an *ex vivo* please italicize ex vivo culture system (Supplementary information, Figure S3A and B). RNA-Seq analysis revealed that the majority of the upregulated genes after EPZ treatment harbored H3K27me3 in the mouse post implantation embryos, and these genes are usually associated with bivalent or repressive states (Supplementary information, Figure S3C). DRGs, such as *T*, *Prdm14*, and *Vax2*, which were enriched for DNAme in the ExE cells and H3K27me3 in specific embryonic cells, e.g., anterior region cells, were uniquely upregulated in the embryonic cells after the treatment of EPZ (Fig. 1f–i; Supplementary information, Figures S3D–F, S4 and Table S3). Moreover, these genes still maintained low expression levels in ExE upon EPZ treatment (Fig. 1g, h; Supplementary information, Figure S3E and F), likely due to the EPZ-insensitive DNA methylation. This distinct feature implies that these genes are indeed repressed by H3K27me3 with a poised state in the embryonic tissues, but are fully silenced by DNA methylation in ExE. Therefore, we propose a model that the differential distributions of DRG-associated DNAme and H3K27me3 contribute to the epigenetic restriction of extraembryonic lineage and spatial regulation of plasticity and development for embryonic lineages, respectively (Fig. 1j).

In summary, we present the epigenomic landscapes of H3K27me3 and DNAme in the mouse post implantation embryos from the E6.5 to E7.5 stages, and identify the distinct features of epigenetic reprogramming for H3K27me3 and DNAme at whole-genome scale during early mouse embryo development. Importantly, we show that the key developmental genes are regulated by H3K27me3 in the embryonic cells and are silenced by DNAme in the ExE cells, which facilitates maintenance of the highly regulated developmental plasticity in the embryonic cells, as well as the permanent restriction of ExE developmental potential. Finally, we functionally validate the existence of distinct epigenetic mechanisms in the post implantation embryos by erasure of H3K27me3. Consistently, a recent study reported that master transcription factors were hypermethylated in extraembryonic lineages, which mirrored the somatic transition to cancer.[10] Here, we further reveal that H3K27me3 is involved in the regulation of ExE-hypermethylated master regulators in embryonic lineages, and distinct distributions of H3K27me3 and DNAme are responsible for the modulation of plasticity maintenance and developmental potential restriction in the embryonic and extraembryonic cells, respectively, which will broaden our understanding of mammalian development and epigenetically regulated human diseases.

Materials and Methods are available in Supplementary information, Data S1. The gene expression, DNA methylation and histone modification data are deposited to Gene Expression Omnibus (GSE104243).

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ADDITIONAL INFORMATION

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