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EVALUATING MITOCHONDRIAL STRESS RESPONSE GENE CLPP-REGULATED DNA METHYLOME DYNAMICS IN FEMALE REPRODUCTIVE

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OBJECTIVE: CLPP (caseinolytic peptidase P) mediates degradation of unfolded mitochondrial proteins to maintain protein hemostasis in response to metabolic and cellular stress. CLPP is required for oocyte and embryo development, and targeted deletion of Clpp results in female infertility and accelerated depletion of ovarian follicular reserve. The aim of the present study was to determine the effect of Clpp-deletion on oocyte and cumulus cell DNA methylome in mature and older mice.

DESIGN: Experimental study.

MATERIALS AND METHODS: Cumulus oophorus complexes (COCs) were collected from 3- and 6-month-old Clpp knockout (Clpp-/-) and wild type (WT) mice (n¼3, per group), 48h after PMSG (5IU) injection Germinal vesicle (GV) oocytes and cumulus cells were isolated, and 5 oocytes and approximately 50 cumulus cells were separately pooled from each mouse for analysis. Whole genome bisulfite sequencing (WGBS) libraries were prepared and sequenced on Illumina's HiSeq 4000 platform. Sequencing reads were pre-filtered and aligned to the mouse reference genome (mm10) using Bismark. DNA methylation levels were determined by the ratio of the number of reads supporting C (methylated) to that of total reads (methylated and unmethylated). Differentially methylated regions (DMRs) were called if DNA methylation level was greater than 80% in one group and less than 20% in the other group with FDR adjusted P <0.05.

RESULTS: Using WGBS, we found that genome-wide methylation level was significantly lower in 3-month Clpp-/-oocytes compared to WT (27.0% vs 52.0%, p<0.01). At 6 month, however, Clpp-/-oocytes had a higher methylation level compared toWT (43.5% vs 25.4%, p<0.01). At 3 months, a total of 4,918 DMRs were identified in Clpp-/-oocytes compared to WT. At 6 month, more DMRs (7,475) were found in Clpp-/- oocytes compared to WT. In cumulus cells, all treatment groups had a similar DNA methylation level, averaging 72.0%, however, the methylation of individual methylated regions was significantly different among different groups. In addition, we found that hyper-methylated regions in both oocytes and cumulus cells were enriched in repeat elements (e.g. LINEs, SINE, LTR), while CGIs, and promoter and enhancer regions were mainly demethylated. Finally, together with RNAseq datasets (Wang et al., 2018), we revealed that promoter methylation was inversely correlated with gene expression of downstream targets that were regulated by Clpp and aging.

CONCLUSIONS: Clpp global deletion resulted in significant changes in genome-wide DNA methylation dynamics of GVoocytes and cumulus cells. Our findings provide new insight into

the role of CLPP in reproduction, and may help understand the potential epigenetic mechanisms mediating infertility and reproductive aging associated with Clpp-deficiency.