Data Article

RNA sequencing data of mouse 2-cell embryos treated with DMSO

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A B S T R A C T

To understand the effect of DMSO in preimplantation embryos, we have treated mouse 1 cell zygotes with DMSO and found that DMSO treatment caused 2 or 4 cell embryonic arrest and altered the acetylation levels of mouse preimplantation embryos. To illustrate the mechanism of DMSO in mouse preimplantation embryos, fertilized zygotes have been treated with 2% of DMSO and then performed RNA-seq analyses. Differentially expressed genes were identified using DESeq2 after adjustment for false discovery rate (FDR q value < 0.05). Gene Set Enrichment Analysis (GSEA) was also performed to identify biological pathways significantly modulated by DMSO. Raw and processed RNA-seq data were deposited and made publicly available on the Gene Expression Omnibus (GEO; GSE124598). The data presented in this article are related to the research paper entitled “DMSO impairs the transcriptional program for maternal-to-embryonic transition by altering histone acetylation”, available in Biomaterials [1].

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Datasets presented here were employed in the main work “DMSO impairs the transcriptional program for maternal-to-embryonic transition by altering histone acetylation” Kang et al., 2020 [1]. Fig. 1 illustrates the experimental procedure. RNA-seq analysis was performed in 2-cell mouse embryos cultured after supplementation of 2% DMSO. The raw data generated from Illumina sequencing were deposited on the Gene Expression Omnibus (GEO) with the reference number GSE124598 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE124598).

RNA-seq analysis was performed in 2-cell embryos with/without DMSO supplementation. In total, 3,742, which is ~20.29% of the total valid genes, genes were differentially expressed in DMSO-treated embryo compared with control embryo with criteria of FDR < 0.05. Of these differentially expressed genes, 1,415 genes were up-regulated, whereas 1,758 genes were down-regulated in DMSO-treated embryo (Fig. 2). DEGs were significantly enriched in total 72 KEGG and REACTOME pathways for DEGs were conducted using ClueGO and CluePedia plug-in in Cytoscape 3.6 software.

Next, we interpreted potential interactive pathways among DEGs associated with epigenetic gene expression, histone modifications (acetylation and methylation) in DMSO treated group using cerebral
Most of DEGs for histone modifications and binding events are significantly depressed at specific and highly characteristic genomic elements and locations in DMSO-treated groups, indicating that DMSO exhibits specific regulatory mechanisms related to regulation of transcription factors, compared with control embryos.

In this study, we proved our hypothesis by RNA-seq analysis to monitor the early embryonic impacts after exposure to DMSO and identified previously unknown underlying molecular mechanisms that explain the DMSO-induced embryonic toxicity, embryo loss, and infertility. Our study suggests for the first time that DMSO exposure induces a significant alteration in gene expression and the functionality of preimplantation embryos via alternations in epigenetic reprogramming. Thus, our findings...
emphasize that the use of DMSO as a standard control test or solvent requires far more cautious consideration, because DMSO can alter cell function by acting as a proteasome or HDAC inhibitor as well as inducing cell toxicity.

2. Experimental design, materials, and methods

2.1. Animals and embryo collection

BDF1 (C57BL/6 × DBA/2; F1; Orient Bio Co. Ltd) mice (8–12 weeks olds) were used for analysis according to guidelines approved by the committee on animal care and use at Konkuk University (IACUC approval number: KU18199). Intraperitoneally injection was carried out in female mice were with pregnant mare’s serum gonadotropin (PMSG; G4527, Sigma Aldrich; 5IU) followed human
chorionic gonadotropin (hCG; CG10, Sigma Aldrich; 5IU) 48 h later, then mated with male mice. Fertilized oocytes with two pronuclei were collected from oviduct at 18–20 h of post hCG injection and each 10 zygotes was cultured in 20ul KSOM (95mM NaCl, 2.5mM KCl, 0.35mM KH2PO4, 0.2mM MgSO4, 10mM Sodium Lactate, 0.2mM Glucose, 0.2mM Sodium pyruvate, 25mM NaHCO3, 1mM Glutamine, 0.01mM Ethylenediaminetetraacetic acid, 5mg/ml Bovine albumine serum) supplemented with 2% DMSO (D2650, Sigma Aldrich) or without. BDF1 embryos with second polar body were collected and cultured in KSOM with/without 2% DMSO for further analysis.

2.2. Library preparation and RNA-seq

Fifty 2-cell embryos from each control and DMSO-treated group were directly subjected to cDNA synthesis using SMARTer® Ultra® Low Input RNA Kit (634940, Clonetech) according to the manufacturer’s instructions. RNA quality was determined using the Agilent Bioanalyzer High Sensitivity DNA kit (5067-4626, Agilent). The synthesized cDNAs with 150-200bp size were used for the preparation of sequencing library using Low Input DNA Library Prep Kit (634946, Clonetech) according to the manufacturer's instructions, and subjected to size selection, followed paired-end reads data were obtained by performing 50 bp sequencing using HiSeq2500 (Illumina).

2.3. RNA-seq data analysis

Reads were pseudomapped using kallisto [2] with default parameters by transcriptome index from FASTA formatted transcriptomes files (GRCm38.re179) of ENSEMBL transcript database (mm10). Transcript abundance of each gene was quantified with the parameters (quant -t -b 100) as transcripts per kilobase million (TPM) using kallisto. Gene-scaled TPM values for each gene transcript were summed by tximport [3] in R/Bioconductor. Differentially expressed gene (DEG) were analyzed by

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**Fig. 4.** Interrelation network among enriched DEGs for epigenetic gene expression and histone modification. Based on GO enrichment test by ClueGO, heat maps and pathway-like visualizations for DEGs that associated with epigenetic gene expression (A), histone methylation (B) and histone acetylation (C) were created using CluePedia plug-in in Cytoscape 3.6 software. Functional relations between DEGs were drawn by colored lines, which represent activation (green), catalysis (purple), inhibition (red), protein modification (light purple) and reaction (black).
| Pathway ID      | Pathway Term                                                                 | adj_pvalue   | No. of Genes | % Genes |
|-----------------|-------------------------------------------------------------------------------|--------------|--------------|---------|
| R-MMU:2262752   | Cellular responses to stress                                                  | 0.00107233  | 92           | 24.02   |
| R-MMU:72702     | Ribosomal scanning and start codon recognition                               | 0.00109672  | 23           | 41.07   |
| R-MMU:1234176   | Oxygen-dependent proline hydroxylation of Hypoxia-inducible factor alpha     | 0.00115378  | 24           | 40.00   |
| R-MMU:72689     | Formation of a pool of free 40S subunits                                     | 0.00117991  | 20           | 44.44   |
| R-MMU:8948751   | Regulation of PTEN stability and activity                                     | 0.00118317  | 25           | 39.06   |
| R-MMU:373076    | Class A/1 (Rhodopsin-like receptors)                                          | 0.00134226  | 20           | 6.25    |
| R-MMU:4641258   | Degradation of DVL                                                            | 0.00147675  | 22           | 41.51   |
| R-MMU:74158     | RNA Polymerase III Transcription                                              | 0.00154192  | 17           | 48.57   |
| R-MMU:76046     | RNA Polymerase III Transcription Initiation                                   | 0.00154192  | 17           | 48.57   |
| R-MMU:6807505   | RNA polymerase II transcribes snRNA genes                                     | 0.00160451  | 27           | 36.99   |
| R-MMU:69275     | G2/M Transition                                                               | 0.00170795  | 50           | 28.57   |
| R-MMU:212436    | Generic Transcription Pathway                                                 | 0.00209786  | 135          | 21.63   |
| R-MMU:453274    | Mitotic G2-G2/M phases                                                        | 0.00214072  | 50           | 28.25   |
| R-MMU:4608870   | Asymmetric localization of PCP proteins                                       | 0.00222022  | 23           | 39.66   |
| R-MMU:5689603   | UCH proteasines                                                               | 0.00278995  | 32           | 33.33   |
| R-MMU:174113    | SCF-beta-TrCP mediated degradation of Emi1                                    | 0.00288664  | 21           | 41.18   |
| R-MMU:8854050   | FBXL7 down-regulates AURKA during mitotic entry and in early mitosis          | 0.00288664  | 21           | 41.18   |
| R-MMU:5687128   | MAPK6/MAPK4 signaling                                                         | 0.00300873  | 26           | 36.62   |
| R-MMU:174154    | APC/C:Cdc20 mediated degradation of Securin                                   | 0.00312854  | 24           | 38.10   |
| R-MMU:56521481  | C-type lectin receptors (CLRs)                                                | 0.003421    | 35           | 31.82   |
| R-MMU:73863     | RNA Polymerase I Transcription Termination                                    | 0.00380234  | 15           | 50.00   |
| R-MMU:1236978   | Cross-presentation of soluble exogenous antigens (endosomes)                 | 0.00388676  | 20           | 41.67   |
| R-MMU:174178    | APC/C-Cdh1 mediated degradation of Cdc20 and other APC/C-Cdh1 targeted proteins in late mitosis/early G1 | 0.00417739  | 25           | 36.76   |
| R-MMU:174184    | Cdc20:Phospho-APC/C mediated degradation of Cyclin A                          | 0.00417739  | 25           | 36.76   |
| R-MMU:351202    | Metabolism of polyamines                                                      | 0.0042154   | 29           | 34.52   |
| R-MMU:68882     | Mitotic Anaphase                                                              | 0.00531307  | 52           | 27.08   |
| KEGG:03008      | Ribosome biogenesis in eukaryotes                                              | 0.00545287  | 36           | 31.03   |
| R-MMU:179419    | APC:Cdc20 mediated degradation of cell cycle proteins prior to satisfaction of the cell cycle checkpoint | 0.00560289  | 25           | 36.23   |
| R-MMU:1234174   | Regulation of Hypoxia-inducible Factor (HIF) by oxygen                       | 0.00579829  | 24           | 36.92   |
| R-MMU:2262749   | Cellular response to hypoxia                                                  | 0.00579829  | 24           | 36.92   |
| R-MMU:5610780   | Degradation of GL1 by the proteasome                                          | 0.00584049  | 21           | 39.62   |
| R-MMU:72086     | mRNA Capping                                                                  | 0.00770714  | 14           | 50.00   |
| R-MMU:112382    | Formation of RNA Pol II elongation complex                                    | 0.00820743  | 21           | 38.89   |
| R-MMU:75955     | RNA Polymerase II Transcription Elongation                                    | 0.00820743  | 21           | 38.89   |
| R-MMU:2555396   | Mitotic Metaphase and Anaphase                                                | 0.00834028  | 52           | 26.94   |
| R-MMU:6807070   | PTEN Regulation                                                               | 0.00889033  | 34           | 31.48   |
| R-MMU:3858494   | Beta-catenin independent WNT signaling                                         | 0.01005271  | 37           | 30.08   |
| R-MMU:2871837   | FCERI mediated NF-kb activation                                                | 0.01119674  | 26           | 35.14   |
| R-MMU:5358346   | Hedgehog ligand biogenesis                                                    | 0.01121237  | 22           | 37.29   |
| R-MMU:5607761   | Dectin-1 mediated noncanonical NF-kb signaling                                 | 0.01133035  | 21           | 38.18   |
DESeq2 [4] in R/Bioconductor with the parameters (baseMean counts >14; false discovery rate (FDR) < 0.05).

### 2.4. Pathway enrichment test and in silico analysis

DEGs were tested for pathway enrichment score in KEGG and REACTOME pathways using ClueGO [5] plug-in in Cytoscape 3.6 (http://www.cytoscape.org). To search potential associations among DEGs specific gene ontology (GO) terms regarding epigenetic gene expression, histone acetylation and histone methylation, ClueGO enrichment test were integrated into CluePedia [6] plug-in in Cytoscape 3.6 and analyzed.
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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dib.2019.105025.

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