SINGLE CELL RNA-SEQ ANALYSIS PROVIDE DEEPER MOLECULAR INSIGHTS INTO ABNORMAL MATURATION OF TUBASTATIN A-TREATED MOUSE OOCYTES

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Introduction: In our previous study, we observed that Tub-A treatment induced an increased level of the acetylation of α-tubulin, and a failure of spindle migration and actin cap formation. Most Tub-A treated oocytes were arrested in an MI-like or a GVBD-like stage and decreased mTOR (spindle formation factor) and mDia1 (inhibitor of actin assembly) in an HDAC6 expression-dependent manner. We wonder if TubA only afftects HDAC6 expression. Therfore the purpose of this study is to clarify the cause of the phenomenon through RNA-seq anaysis.

Materials and methods:

Tubastatin A treatment: Tubastatin A HCl (Catalogue no. 27108) was obtained from BPS Bioscience (San Diego, USA). The compound was dissolved in DMSO and further diluted in saline to the 10 and 20 μM concentration. Oocytes in the control group were incubated with the same amount of solvent alone (DMSO) for 12 h.

RNA-sequence analysis: The library was prepared using an Illumina NextSeq (Illumina) according to the manufacturer's instructions. The gene annotation of the mouse reference mm10 from UCSC genome (https://genome.ucsc.edu) in GTF format was used as gene models, and the expression values were calculated in Fragments Per Kilobase of transcript per million fragments mapped (FPKM) unit.

Results and discussion: In the Tub-A treated group, 3665 genes were differently expressed, compared to untreated group (GV or MII stage oocytes). Of them, 1,547 and 2,118 genes in TubA treated group are up- or down-regulated. Also, 718 (4.2%), 723 (4.3%), and 294 (1.7%) genes were specifically expressed at GV, MII, and Tub-A-treated oocytes, respectively. Of note, p53 signaling pathway, MAPK signaling pathway, Wnt signaling pathway, and Notch signaling pathway related gene expressions in TubA treated group are significantly increased, whereas metabolism, DNA replication, and oxidative phosphorylation related gene expression are significantly decreased. Furthermore, TubA treated group showed significant alternations in histone methyltransferase-related gene expression, indicating that abnormal meiotic maturation of mouse oocytes by TubA treatment is caused by combined inhibitory effects such as HDACs and Sirtuins, but not HDAC6 inhibitory effect alone. RNA-seq and in silico pathway analysis demonstrate the potential for using mouse oocytes as an in vitro platform for the systematic validation of chemotherapeutic targets.