American Society for Reproductive Medicine 2018 Scientific Congress & Expo
October 6 to 10, 2018 ● Denver, Colorado, USA

Title:

THE MORULA STAGE TRANSCRIPTOME IS CHARACTERIZED BY MARKED UPREGULATION OF GENES THAT MEDIATE KEY MITOCHONDRIAL FUNCTIONS

Authors:

L. Sekhon,[1,2] K. Allette,[3] E. Ellis,[3] Y. Wang,[3] J. Lee,[1] C. Briton-Jones,[1] E. Schadt,[3,4] R. P. Sebra,[3,4] A. B. Copperman[1, 2, 4]

Affiliations:

1. Reproductive Medicine Associates of New York, 635 Madison Ave 10th Floor New York, New York, United States, 10022

2. Obstetrics, Gynecology and Reproductive Science, Icahn School of Medicine at Mount Sinai, Klingenstein Pavilion 1176 Fifth Avenue 9th Floor New York, New York, United States, 10029

3. Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, 1425 Madison Ave, New York, NY 10029

4. Sema4, 333 Ludlow Street, South Tower, 3rd floor, Stamford, CT, United States, 06902

Objective:

The application of RNA sequencing to human preimplantation embryos may unlock insights into the transcriptional programs mediating early embryogenesis. While the transcriptome of cleavage-stage embryos has been widely studied in the animal model, there is little literature related to molecular analysis at and beyond the morula stage in human embryos. Compaction involves cellular polarization and differentiation, in preparation for blastulation. Gene pathways involved in energy mobilization and waste disposal have been demonstrated to be key to the cleavage-to-embryo stage transition (Hasegawa et al., 2015). The dependence of blastocoel cavity formation on cellular Na/K-ATPase activity is supported by a recently published report that peak mitochondrial oxygen consumption in embryos occurs at the

morula stage (Hashimoto et al., 2017). The study aimed to broaden the understanding of early human embryogenesis by characterizing differential gene expression across the three major stages of preimplantation development.

Design:

Prospective cohort study on human embryos donated for research.

Materials and Methods:

The study included patients who donated fresh embryos at various stages of development during an IVF cycle between January, 2016 and June, 2016. Embryos underwent routine evaluation from day 3 to 7 of extended culture, and were classified into 3 developmental stage groups: cleavage stage, morula stage, and blastocyst stage. Embryos were biopsied, and approximately 2-4 cells were removed for aneuploidy screening by next generation sequencing (NGS) using the ReproSeq assay to assess CNVs. The remaining cells of the embryo were designated for RNA Sequencing. Read counts per gene were summed across embryo cohorts and normalized using the median of ratios. Differential gene expression between embryo cohorts was calculated using DESeq2, in order to estimate variance-mean dependence and evaluate differential gene expression using a negative binomial distribution. A likelihood ratio test was used to account for heterogeneity due to patient, batch, and ploidy and growth status (arrested/ongoing). Pathway analysis was performed and grouped according to major cellular functions (Table 1). The adjusted threshold for significance was $p<0.05$.

Results:

Differential gene expression was compared among 29 cleavage stage, 9 morula, and 43 blastocyst stage embryos. Of the 20,553 protein-coding transcripts interrogated, 3 were found to have significant differential expression between the developmental stages (POLR1D, SULT2A1, and CSAG3).

Transcriptional activity, as evidenced by POLR1D expression (encodes RNA Polymerase I/III), peaked at the morula stage. The morula stage transcriptome was characterized by marked upregulation (Table 1), of genes, including those involved in key mitochondrial processes, such as: CISD1(an iron-containing outer membrane protein that is protective against mitochondrial injury and cell death); ISCA1 (an iron-sulfur cluster machinery component involved in mitochondrial respiration); MFN2 (mediates the dynamic balance between fusion and fission that determines mitochondrial morphology); MRPL42 (encodes mitochondrial ribosomes for protein synthesis); and SUCLG1 (catalyzes the conversion of succinyl CoA and ADP/GDP to ATP/GTP).

Conclusion:

Differential gene expression across the various stages of preimplantation embryo development provide insights into the critical functions required for early embryogenesis. Transcripts mediating major mitochondrial functions appear to play an important role in compaction and preparation for cell differentiation and blastulation. Enrichment in processes related to mitochondrial function in the morula may reflect the switch from anaerobic glycolysis to aerobic metabolism which occurs by the blastocyst stage. A deeper understanding of the molecular pathways that drive the biological cleavage-to-blastocyst transformation may serve as the basis for future advances in embryo culture technique that optimize energy generation, minimize stress, and ultimately improve blastulation rates and human reproductive outcome.

Table 1:

Differentially expressed genes in morula stage versus cleavage and blastocyst stage embryos. The adjusted threshold for significance was p<0.05.

	Downregulated			Upregulated		
Pathways	**Gene**	**Log2 fold Δ**	**Adjusted**	**Gene**	**Log2 fold Δ**	**Adjusted p**

			p value			value
Mitochondrial function				**CISD1**	3.29	0.02
				ISCA1	3.36	0.045
				MFN2	4.41	0.045
				MRPL42	3.08	0.048
				SUCLG1	4.44	0.046
Anti-apoptotic	**FBXL2**	-5.39	0.02	**MAST2**	6.27	0.02
Lysosomal function				**CLN5**	6.77	0.045
Transcription mediating				**POLR1D**	3.18	0.003
				POLR2L	3.72	0.046
Post-translational modification	**NMT2**	-3.91	0.045			
Uncategorized function				**C4orf3**	3.96	0.02

References:

1. Hasegawa Y, Taylor D, Ovchinnikov DA, Wolvetang EJ, de Torrente L, Mar JC. Variability of gene expression identifies transcriptional regulators of early human embryonic development. PLoS Genetics 2015; 11(8): 1-32.

2. Hashimoto S, Morimoto N, Yamanaka M, Matsumoto H, Yamochi T, Goto H, Inoue M, Nakaoka Y, Shibahara H, Morimoto Y. Quantitiative and qualitative changes of mitochondria in human preimplantation embryos. JARG 2017; 34: 573-80.