

THE EPIGENETIC REGULATION OF MICRORNAS AND RELATED PATHWAYS IN PREIMPLANTATION EMBRYO DEVELOPMENT

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Purpose: Epigenetic regulation is very important for in early embryo development for it can affect genomic imprinting, gene reprogramming and gene expression. Current studies have indicated that epigenetic regulation influences gene control mainly through DNA methylation, histone modification and microRNAs (miRNAs). To further investigate the gene regulation during preimplantation stages, we would examine the functions of microRNAs in both epigenetic control and gene regulation during early embryo development.

Materials and methods: Animal and embryo collection. The 5 weeks old ICR mice were superovulated and mice embryos were collected and cultured in human tubal fluid medium containing 0.3% of bovine serum albumin.

Drug treatment. The miR profiling of ICR mice embryos from morula to blastocyst with or without Dnmt inhibitors. Embryos treated 24hrs with or without Dnmt inhibitors were collected for total RNA extraction. MicroRNAs qPCR. Total RNA was extracted by using RNeasy Lysis Reagents. Primer sets are designed to amplify mature microRNAs. Products were amplified from 10 ng of total RNA samples with the TaqMan MicroRNA Assay, Quantitative-PCR machine, and software from Applied Biosystems. **Experimental analysis** The miR targets were predicted with some modification from the comprehensive databases of experimentally supported animal miR targets software including pathway analysis for mouse, MiRNAmapper and Clone gene converter.

Results: The embryo developments were significantly and dose-dependently inhibited after 24 h treatment with 1-5 M 5-aza-2'-deoxycytidine during morula to blastocyst stage. Whereas low dose of 5-aza-2'-deoxycytidine (1M) could arrest embryo development and high dose of treatment (5M) could induce embryo fragmentation and cell lysis. Using RT-qPCR with multiplex primers set, we have identified that a set of 65 microRNAs have significantly different expression level between morula, blastocyst and 5-aza-2'-deoxycytidine treated embryos. Thousands of targets were predicted by the epigenetic regulated microRNAs. We further compared the microRNAs target genes with microarray data of embryos during morula to blastocyst, several genes involved in cell cycle, cell adhesion, differentiation, and several transcriptional factors were identified, including cyclin A2, cyclin G1, CDC42, klf4, Dnmt3a, ESR1, Itga5. The genes were grouped by pathway analysis softwares and data showed that these miR-targets were involved in TGF-beta signaling pathway, MAPK signaling pathway, and tight junction.

Conclusion: This study we show the miR profiles in early embryo development and also identified the epigenetic-regulated specific miRs, further predicting by using a software-assisted approach demonstrated that several growth-related genes and pathways were targeted. This study might improve our knowledge of epigenetic-regulated miRs in early embryo growth and cell differentiation during pre-implantation embryo development; it should be helpful for further works in studying the imprinting and re-programming regulation in reproductive medicine.