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## 大會手冊暨論文摘要

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地點：台北國際會議中心101CD,103室

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## 以附基因調控研究微小核糖核酸於著床前胚胎發育過程中之角色 **Epigenetic Regulation of MicroRNA in Pre-implantation Embryo Development**

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**Introduction:** During preimplantation development, epigenetic modifications such as DNA methylation, histone modifications, and/or microRNA regulation are involved in the regulation of imprinted and non-imprinted genes. This epigenetic reprogramming is likely to be needed for totipotency, correct initiation of embryonic gene expression, and early lineage development in the embryo. According to mouse cDNA microarray analysis, we have identified several epigenetic regulation factors related to DNA methylation or histone acetylation which are highly regulated during pre-implantation embryo development. Thus, we concluded that these genes may play an important role in epigenetic regulation during early embryo development process and some important miRs could be regulated and play important roles in pre-implantation embryo development.

**Materials and Methods:** 5 weeks ICR mice were superovulated and embryos were collected and cultured in human tubal fluid medium. Embryos treated 24hrs with or without Dnmt inhibitors and HDAC inhibitors were collected for total RNA extraction. Products were amplified from 10 ng of total RNA samples with the TaqMan MicroRNA Assay. 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 convertor.

**Results:** By using real time RT-PCR analysis, the dynamic gene expression changes of DNA methylation or histone deacetylation-related factors have been identified from 2-cell stage to hatched embryo. The Dnmt3b and SIN3b showed significantly increased after morula stage, whereas the HD-1 and SIN3a were decreased. The embryo developments were significantly and dose-dependently inhibited after 24 h treatment with 1-5  $\mu$ M 5-aza-2'-deoxycytidine during morula to blastocyst stage. Whereas low dose of 5-aza-2'-deoxycytidine (1  $\mu$ M) could arrest embryo development and high dose of treatment (5  $\mu$ M) could induce embryo fragmentation and cell lysis. Using RTQRT-PCR with multiplex primers set, we have identified that miR-24, 30a, 99b, 150, 200c, and 199a could be highly regulated. Hundreds of predicted targets of these miRs indicated that several important genes involved in cell cycle, cell adhesion, differentiation, and several transcriptional factors might be regulated by the epigenetic-related miRs. These genes were further grouped by the pathway enrichment analysis and the data showed that these miR-targets were involved in TGF-beta signaling pathway, MAPK signaling pathway, Notch signaling pathway, purine and pyrimidine metabolism, and tight junction.

**Conclusions:** In 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.