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Effects of uterine cells-conditioned media on expression of DNMT3β and DNMT3C in mouse embryos cultured in a microfluidic device

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KEYWORDS: microfluidics, in vitro culture, real-time PCR

INTRODUCTION:

DNA methylation is an epigenetic mechanism that plays an important role in early embryo development in mammals. We hypothesize that the de novo DNA methyltransferase genes, DNMT3β and DNMT3C, can be affected by medium composition during in vitro mouse embryo development. Herein, we describe the culture of murine embryos in a microfabricated polydimethylsiloxane (PDMS) device and we investigated the effect of cells-conditioned media on gene expression of individual blastocysts by qPCR.

METHODS: The microfluidic device was fabricated in PDMS using traditional soft-lithographic technique. Groups of 12, 1-cell murine embryos (B6C3F1xB6D2F1 strain, EmbryoTech, USA) were cultured in mouse uterine epithelial cells-conditioned media into a microfluidic device (n=15 devices, 180 embryos). Control embryos were cultured in the device using control media (n=15 devices, 180 embryos). We analysed expression of two genes involved in the de novo DNA methylation process, DNA methyltransferase 3β (DNMT3β) and DNA (cytosine-5-)-methyltransferase 3c (DNMT3C), in blastocysts cultured in cells-conditioned media compared with those cultured in control media. To determine transcript abundance of the selected genes we performed qPCR of cDNAs generated from individual, stage matched late-blastocysts cultured in the microfluidic device.

RESULTS: From the analysis of qPCR results we observed that both DNMT3β (n=12, p=0.006) and DNMT3C (n=12, p=0.048) were highly expressed in blastocysts cultured in cell-conditioned media compared with those cultured in control media. The upregulation of DNMT3β and DNMT3C suggests that the use of cells-conditioned media might be an efficient approach to improve quality of embryos produced in vitro. However, the expression pattern of those genes may vary during embryonic development and our findings need to be confirmed by investigating transcript abundance in blastocyst from different stages.

CONCLUSIONS: We conclude that mRNA relative expression levels of DNMT3β and DNMT3C were significantly affected by the use of cells-conditioned media during preimplantation in vitro development of mouse embryo in a microfluidic device. Further studies will be required to elucidate the effect of this variation in mouse embryo development.