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Analysis of gene expression in single human oocytes and preimplantation embryos ☆

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Abstract

Little is known about the gene expression in human oocytes and early embryo development because of the rare availability of the materials. The recent advancement of biotechnology has allowed one to analyze the gene expression in single human oocytes and preimplantation embryos. Gene expression of human lactate dehydrogenase isozymes (LDH-A, LDH-B, and LDH-C) and small ubiquitin-like modifier isoforms (SUMO-1, SUMO-2, and SUMO-3) in four oocytes, two 4-cell and three 8-cell embryos was studied using the reverse transcription-polymerase chain reaction. The mRNAs for *SUMO-1*, *SUMO-2*, *SUMO-3*, and *LDH-B* (heart) were detected in all of oocytes, 4- and 8-cell embryos. The mRNA for *LDH-A* (muscle) was detected in two of four oocytes and one of three 8-cell embryos. However, the mRNA for testis-specific *LDH-C* was not detected at all as expected. A cDNA microarray containing 9600 cDNA spots was used to investigate differential expression of human genes in oocyte, 4-cell and 8-cell embryos. The expression of 184, 29, and 65 genes was found to have a value more than twofold above the median value of all genes expressed in oocyte, 4- and 8-cell embryos, respectively, indicating that the expression of some zygotic genes had already occurred at 4-cell embryo.

Keywords: Human; Oocytes; Preimplantation embryos; RT-PCR; cDNA microarray

Mammalian oocyte maturation and early embryo development require the appropriate expression of many genes at different developmental stages. In oocytes, some transcripts are required for oocyte-specific processes and metabolism, while others are presumably stored for use during early embryonic development, prior to the activation of zygotic expression. Fertilization releases the oocyte from cell cycle arrested in second meiotic metaphase and initiates the activation of the embryonic genome. In mice, significant changes in gene expression were found during the early developmental processes, and the stage-specific expressed genes actively promote the advancement of embryos from one stage to the next [1–3]. The gene expression profiling of human oocytes and early embryo development has been difficult to study due to the rare availability of the materials and the associated ethical considerations. However, the recent advancement on linear amplification of very small amounts of RNA samples for successful microarray detection has allowed one to analyze gene expression profiles among single human oocytes and preimplantation embryos obtained through in vitro fertilization (IVF) clinics [4].

Energy metabolism is a key feature of human preimplantation embryo development. Developmental abnormalities may be caused by defective energy metabolism [5], and thus it is important to assess the gene expression of metabolic enzymes in preimplantation embryos. Lactate and pyruvate are the preferred nutrients for the cleavage-stage embryo of several mammalian species, including the human [6]. The interconversion of lactate and pyruvate is

☆ *Abbreviations*: IVF, in vitro fertilization; LDH, lactate dehydrogenase; RT-PCR, reverse transcriptase-polymerase chain reaction; SUMO, small ubiquitin-like modifier.

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catalyzed by lactate dehydrogenases (LDH) with nicotinamide adenine dinucleotide as a coenzyme. Mammalian LDH isozymes are encoded by three different genes: *LDH-A* (muscle), *LDH-B* (heart), and *LDH-C* (testis). Five tetrameric LDH isozymes present in somatic tissues are various combinations of LDH-A and LDH-B subunits, whereas homotetrameric LDH-C isozyme is found only in testis and spermatozoa. LDH-A/B/C isozymes possess distinct catalytic properties [7,8].

Human small ubiquitin-like modifier (SUMO) proteins have recently been identified and they are involved in protein trafficking and targeting through posttranslational modification [9]. SUMO-1, SUMO-2, and SUMO-3 proteins were shown to be located on the nuclear membrane, in the nucleus, and in the cytosol, respectively [10]. The sumoylation participates in a number of cellular processes such as nuclear transport, transcriptional regulation, apoptosis, and so on.

In the present study, the expression of human *LDH-A/B/C* and *SUMO-1/2/3*, as well as β-actin, was first analyzed using reverse transcription-polymerase chain reaction (RT-PCR) in order to assess the quality of RNAs isolated from single oocytes, 4- and 8-cell embryos. A cDNA microarray was then used to survey differential expression profiles of human genes in single oocytes, 4- and 8-cell preimplantation embryos, and some zygotic genes were found to be expressed at 4-cell embryo.

Materials and methods

Human oocytes and embryo collection. Four oocytes, two 4- and three 8-cell preimplantation embryos, which were surplus to requirement for infertility treatment, were obtained with the IRB approval from Kaohsiung Medical University and the patients' consent at Profertile IVF Center. Oocytes were treated with hyaluronidase type VIII (80 iμ/ml) to get rid of the surrounding granulosa cells and checked for the first polar body to make sure matured oocytes. Embryos were treated with acid Tyrode's solution for 30 s to free sperm and other cell contamination.

RNA isolation and cDNA synthesis. Single oocytes and embryos were washed with PBS twice and then transferred to Eppendorf in 2 μl PBS, 2 μl lysis buffer [0.5% NP-40 (Vysis, Dowers Grove, IL, USA), 20 mM DTT (Invitrogen, Carlsland, CA, USA)], and 1 μl of 0.5 μg/μl oligo$(dT)_{12–18}$ primer (Invitrogen). Samples were incubated for 5 min at 65 °C to lyse the cells, to release RNAs, and to anneal oligo$(dT)_{12–18}$ primer with RNAs. The cDNA synthesis was carried out by using SURPERSCRIPT One-Step RT-PCR Kit (Invitrogen). For the reverse transcription step, the whole 5 μl of the resuspended RNAs was incubated for 60 min at 42 °C, then 15 min at 72 °C in 50 μl of reaction mixture containing 25 μl of 2×Reaction Mix (Invitrogen) and 1 μl of RT/PLATINUM *Taq* Mix (Invitrogen).

Polymerase-chain reaction. Polymerase-chain reaction (PCR) primers of complementary DNA were designed using Primer 3 web site software (http://www.broad.mit.edu/cgi-bin/primer/primer3_www.cgi). PCR primer sequences for *LDH-A/B/C*, *SUMO-1/2/3*, and β-actin, as well as the sizes of PCR products, are presented in Table 1. PCR amplification of the cDNAs was carried out using 4 μl from the above 50 rmul RT cDNA reaction mixture, added to 25 μl of reaction mixture containing 1×PCR buffer (with 2.0 mM $MgCl_2$), 0.2 mM dNTP, 0.4 μM each primer, and 2.5 U *VioTaq* DNA polymerase (Viogene, Taiwan). The PCR conditions were as follows: initial denaturation for 5 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 56 °C, and 1 min at 72 °C, and final extension for 7 min at 72 °C. The 7.5 μl of the amplified PCR products was electrophoresed on a 3% agarose gel.

Table 1
The PCR primer pairs used to amplify LDH-A/B/C, SUMO-1/2/3, β-actin, and the size of their products

Genes	Primers		Size of products (bp)
LDH-A	F	5′GGCCTGTGCCATCAGTATCT3′	189
	R	5′GCCGTGATAATGACCAGCTT3′	
LDH-B	F	5′AGGATTCATCCCGTGTCAAC3′	169
	R	5′CCCACAGGGTATCTGCACTT3′	
LDH-C	F	5′CCTCTTGGGCTATTGGACTG3′	199
	R	5′GCCTCCTCCTCAGAATTCAA3′	
SUMO-1	F	5′CAGGAGGCAAAACCTTCAAC3′	293
	R	5′TCCATTCCCAGTTCTTTTGG3′	
SUMO-2	F	5′GGATGGTTCTGTGGTGCAGT3′	157
	R	5′TTCCAACTGTGCAGGTGTGT3′	
SUMO-3	F	5′CCAAGGAGGGTGTGAAGACA3′	212
	R	5′TCTCAGCTGTGCTGGAGTGT3′	
β-actin	F	5′GGACTTCGAGCAAGAGATGG3′	234
	R	5′AGCACTGTGTTGGCGTACAG3′	

cDNA microarray. 28.5 μl of the cDNAs present in above 50 μl RT reaction mixture purified by Microarray Target Purification Kit was used as templates to amplify cDNA by Microarray Target Amplification Kit (Roche Applied Science, Indianapolis, ID, USA). The complementary RNAs (cRNAs) were synthesized from the purified 200 ng cDNA by Microarray RNA Target Synthesis Kit (Roche Applied Science). The purified 5 μg cRNAs from single oocyte, 4- and 8-cell embryos were analyzed by the cDNA Microarray Core Facility for Genomic Medicine at National Taiwan University. The cDNA microarray contains 9600 PCR-amplified cDNA fragments from Integrated Molecular Analysis of Genomes and their Expression [IMAGE] human cDNA clones, with each spot representing a putative gene cluster with an assigned gene name in the Unigene clustering. The details of cDNA microarray experiments, including probe preparation, hybridization, and color development, were described previously [11,12]. After hybridization and color development of cDNA microarrays, the spots of good quality having a signal over background at least 1.5-fold were included in the further analysis, and the raw data were normalized per gene to median value.

Results

RT-PCR analysis of LDH-A/B/C and SUMO-1/2/3 gene expression in single human oocytes and preimplantation embryos

The gene expression of lactate dehydrogenase isozymes (LDH-A, LDH-B, and LDH-C) and small ubiquitin-like modifier isoforms (SUMO-1, SUMO-2, and SUMO-3), as well as β-actin, in four oocytes, two 4-cell and three 8-cell preimplantation embryos was analyzed using RT-PCR (Fig. 1). The mRNAs for *SUMO-1*, *SUMO-2*, *SUMO-3*, and *LDH-B* (heart) were detected in all of oocytes, 4- and 8-cell embryos. The mRNA for *LDH-A* (muscle) was detected only in two of four oocytes and one of three 8-cell embryos. The mRNA for testis-specific *LDH-C* was not detected at all as expected. The β-actin was used as positive control.