

착상 전 생쥐배아에서 Id 유전자의 발현

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Expression of Ids in Preimplantation Mouse Embryos

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Objective: The Id family of helix-loop-helix proteins are thought to affect the balance between cell growth and differentiation by negatively regulating the function of basic-helix-loop-helix (bHLH) transcriptional factors. The aim of this study was to investigate the expression pattern of Ids (Id-1,-2,-3, and -4) in preimplantation mouse embryos at mRNA and protein levels.

Methods: Oocytes and preimplantation embryos were collected from reproductive organs of female ICR mice following superovulation. RT-PCR was performed to investigate the mRNA expression patterns of Id genes and their protein were localized by immunofluorescence analysis.

Results: Id-1 and Id-3 mRNAs were strongly expressed at the germinal vesicle (GV) oocyte and the blastocyst stages. Id-2 mRNA was expressed throughout preimplantation embryo development, but Id-4 was not expressed. Immunofluorescence showed that Id-1 and Id-2 were predominantly localized in cytoplasmic region, but the immunofluorescence signal of Id-3 was weak throughout preimplantation embryo development.

Conclusion: These data show for the first time that Ids are expressed in preimplantation mouse embryos and suggest that Ids may play an important role in early preimplantation embryo development and uterine physiological changes.

Key Words: Id, Immunofluorescence, Mouse, Preimplantation embryo

Basic helix-loop-helix domain (bHLH) 가

HLH

DNA

bHLH

dominant negative HLH (dnHLH)

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bHLH HLH motif가 basic amino acid가 dnHLH DNA bHLH Id DNA (Inhibitor of DNA binding) (inhibiting differentiation) Id family (myogenesis),⁸ (lymphopoiesis),¹⁰ (sial cell development)¹² Id 가 bHLH Id 가 Id-2 (mammary epithelial cells) (spermatogenesis)^{13,14} Id-1 Id¹⁵ Id¹⁶ Id Id carrier RNA (Takara, Shiga, Japan) (2×10⁵ copies/μl) 가 5 isopropanol (Sigma) 가 14,000 rpm, 4 , 30 75% ethanol 1 ml 가 14,000 rpm, 4 , 15 0.1% DEPC

가 , 가 ICR 6~8 , 10 (germinal vesicle oocyte) 6 5 IU pregnant mare's serum gonadotropin (PMSG; Sigma, St. Louis, MO, USA) 46 , 19 gauge (MII oocyte) 8 5 IU PMSG , 48 5 IU human chorionic gonadotropin (hCG; Sigma) hCG 14 - , hyaluronidase (Sigma) 1 가 8 PMSG hCG post-hCG 20, 48, 56, 65, 72, 96 , 2- , 4- , 8- , 2. Total RNA RT - PCR 0.1% diethyl-pyrocabonate (DEPC; Sigma)가 3 Ca²⁺, Mg²⁺-free phosphate buffered saline (PBS) , 0.1% DEPC가 3 Trizol (Invitrogen, Carlsbad, CA, USA) 1.5 ml RNA RNA carrier RNA (Takara, Shiga, Japan) 1 μl (2×10⁵ copies/μl) 가 , 50 μl chloroform (Sigma) 가 5 14,000 rpm, 4 , 15 isopropanol (Sigma) 가 . 1 14,000 rpm, 4 , 30 75% ethanol 1 ml 가 14,000 rpm, 4 , 15 0.1% DEPC

1. 12 , 12 0.1% DEPC

Table 1. Primers designed for amplification of Id genes

Gene		Forward (F) and Reverse (R) primer sequences	Product size (bp)
Id-1	F	GATCATGAAGGTCGCCAGTG	539
	R	TCCATCTGGTCTCAGTGC	
Id-2	F	GTGACCAAGATGGAAATCCT	703
	R	TTTATTTAGCCACAGAGTAC	
Id-3	F	GAAGGCGCTGAGCCCGGTGC	523
	R	GTTCGGGAGGTGCCAGGACG	
Id-4	F	GCGATATGAACGACTGCTAC	383
	R	TCACCCTGCTTGTTACGGC	
β-actin	F	GTGGGCCGCTCTAGGCACCAA	616
	R	CTCTTTGATGTCACGCACGATTTC	

24 μl 가 75 10 1 2 ,
 (reverse transcription) PBS 3 FITC-conjugated goat anti-rabbit
 30 RNA IgG (Santa Cruz) 2
 PBS 2
 (polymerase chain reaction) Hoechst33342 (Sigma) counterstaining ,
 가 (Table 1), (Olympus BX-40, Tokyo, Japan)
 94 30 (denatu-
 ration), 60 30 (annealing), 72
 30 (extension)
 1.2% 가 1. RT - PCR Id
 , UV cDNA , RNA Id
 β-actin
 positive control (Figure 1). Id-1 Id-3
 3. , 2-
 10% sucrose가 가 2% paraformaldehyde 가 Id-2
 15 , PBS 3
 3% BSA가 0.4% (v/v) , Id-1, Id-3
 Triton X-100 10 permeabilization 가
 , 1 Id-4
 2% fetal bovine serum (FBS; Gibco BRL, Rockville,
 MD, USA) 가 PBS 30 2.
 1:200 Id-1, Id-2, Id-3
 (Santa Cruz biothechnology, Santa Cruz, CA, USA)

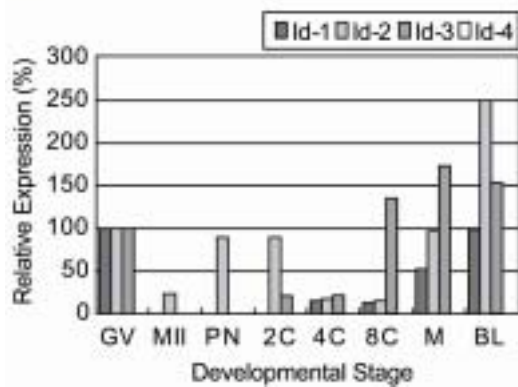
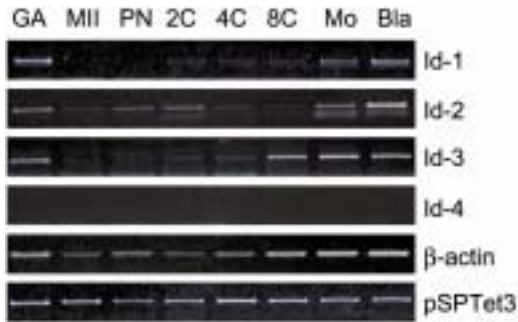


Figure 1. Temporal expression patterns of Id family genes during oocyte growth and preimplantation development in mouse. The experiment was performed twice and the data, which are expressed relative to the amount present in the GV oocyte, are expressed as the mean \pm range. GV, germinal vesicle oocyte; MII, metaphase II oocyte; PN, pronuclear stage embryo; 2C, 4C, 8C, M and BL represent two-cell, four-cell, eight-cell, morula, and blastocyst stage embryos, respectively.

Id (Figure 2). 3 Id 10~15 Id-1 (Figure 2A1-2A6). Id-2 Id-1 2-, 4-, 8- (Figure 2B1-2B6). Id-3

(Figure 2C1-2C6).

Id dominant negative HLH (dn-HLH) HLH motif가 DNA basic amino acid가 bHLH bHLH Id Reese cDNA microarray 가 Id-1 가 (attachment) Watanabe Id-2 17,18 Hong Id-1 Id-2 가 16 Nah laser capture microdissection Id-1 Id-2 Id-3 zygotie gene activation 2- 가

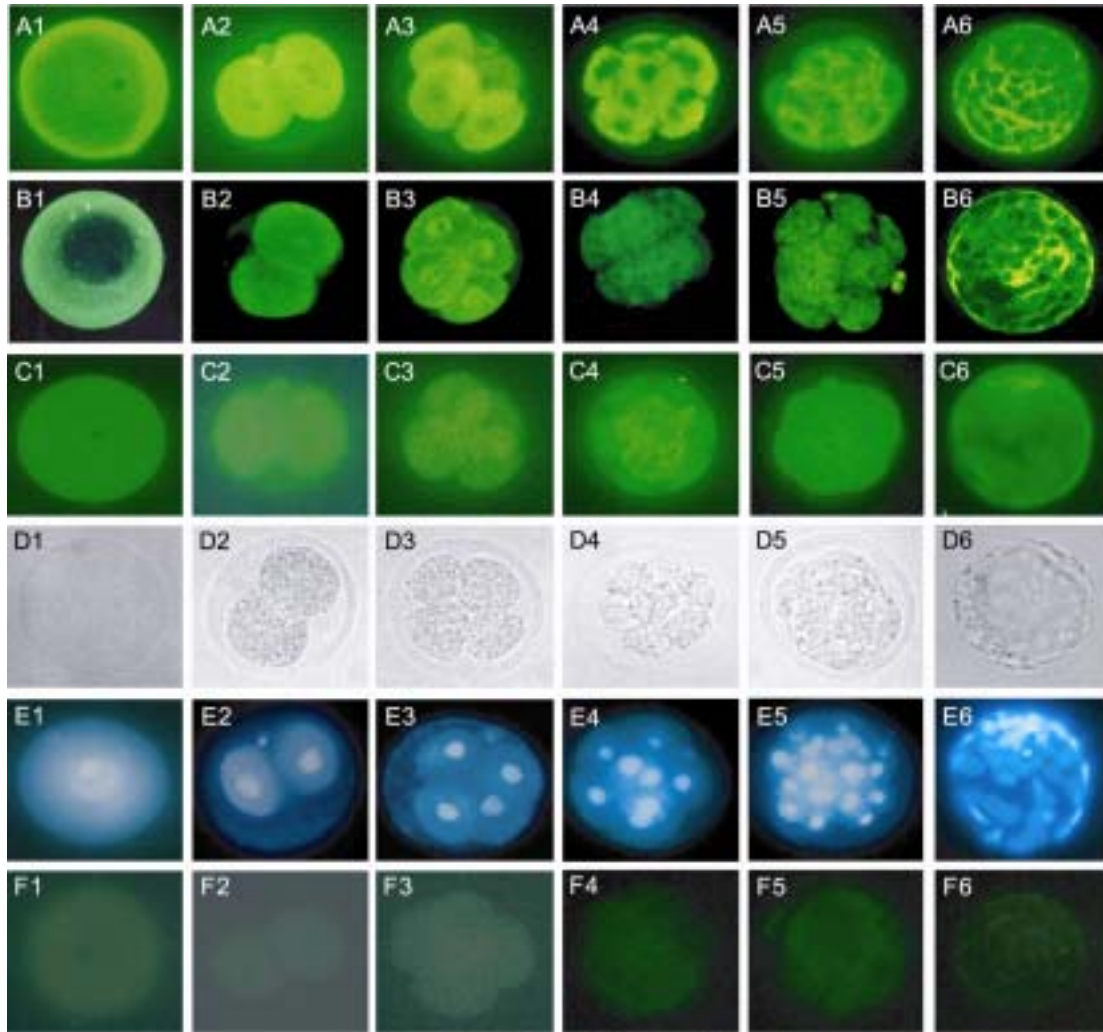


Figure 2. Immunostaining of Id-1, -2, and -3 proteins during oocyte growth and preimplantation embryo development in mouse. Embryos at various developmental stages were collected and immunostained with Id-1, -2, and -3 antibodies (A1-6, B1-6, C1-6). The signals were detected with a FITC-conjugated secondary antibody and the nucleus was stained with Hechst33342 (E1-6). For negative control, Id-1, -2, and -3 antibodies were not treated (F1-6) and brightfield photograph was shown in D1-6. A1-F1, germinal vesicle oocyte; A2-F2, two-cell; A3-F3, four-cell; A4-F4, eight-cell; A5-F5, morula; A6-F6, blastocyst. The signals were visualized using Olympus fluorescence microscope ($\times 400$).

가 , Id-4
 Id-1 Id-2가 Id-1 Id-2
 Id-3 Id-1 Id-2 Id 가 가
 2-, 4-, 8-

(angiogenesis) Id 가 , Id-1 Id-3 (neuroectoderm) , (tumour xenograft) Id-1 가 , Id (endothelial cells) (VEGF, vascular endothelial growth factor) 가 , Id 가 , Id dnHLH HLH , Id Id-1, Id-2, Id-3, Id-4 4 Id family 가 ^{5,6,7} (myogenesis),⁸ (myelopoiesis),⁹ (lymphopoiesis),¹⁰ (bone morphogenesis),¹¹ (kidney glomerular mesangial cell development)¹² Id 가 bHLH , Jen Chaudhary Id family , ^{21,22} Id family (nonredundancy) Id (Id-1, -2, -3, -4) , 가 , 가 Id , Id

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