

Heparin-Binding Epidermal Growth Factor가 MMP-9 ATPase α -subunit

1,2, 2, 1, 2, 2, 2, 1 2

Effects of Heparin-Binding Epidermal Growth Factor on the expression of MMP-9 and ATPase α -subunit mRNA in the Mouse Embryo

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Objectives: The purpose of this study was to evaluate effects of heparin-binding epidermal growth factor (HB-EGF) on the rate of blastocyst formation and hatching in the mouse embryos and the expression of matrix metalloproteinase-9 (MMP-9) and ATPase α -subunit mRNA.

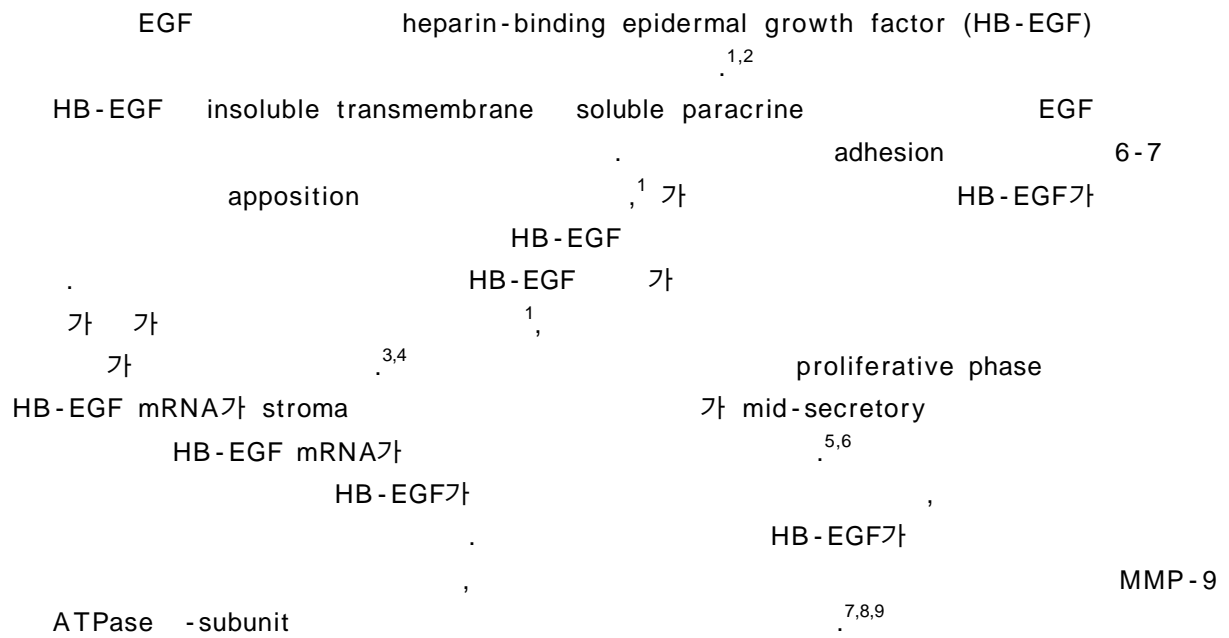
Methods: Late 2-cell mouse embryos was cultured for 72 hours in HTF medium containing with 1, 10, and 100 ng/ml HB-EGF. The mRNA expression level of MMP-9 and ATPase α -subunit was detected by reverse transcription-polymerase chain reaction(RT-PCR).

Results: The rate of hatching was significantly higher ($p<0.05$) in group containing with 1 ng/ml HB-EGF than other groups. Also, the rate of hatched blastocyst was significantly higher ($p<0.05$) in 10 ng/ml. The mRNA expression level of MMP-9 mRNA was not shown any difference among groups, but ATPase α -subunit was higher than other groups.

Conclusions: Taken together these results suggest that HB-EGF has the positive effect to promote the blastocyst formation and hatching process and influences the blastocoel expansion by promoting the ATPase mRNA expression in the mouse embryos.

Key Words: ATPase α -subunit, Blastocoel, HB-EGF, MMP-9, RT-PCR

(synchronization)



1.

14 , 10 가 , 가
(ICR starin) . 8-10 , 10-12

5 IU pregnant mare's serum gonadotropin
(PMSG, Sigma) 48 5 IU human chorionic gonadotropin (hCG, Sigma)
, hCG 48

2- (SZ-40, Olympus)
(flushing)

2.

(60×15 mm, Falcon) (50 μ)
light mineral oil (Sigma) 37 , 5% CO₂ 95% 가 100% 가
(TC-2323, Sheldon)
5% SSS (Synthetic serum substitute, Irvine)가 가 HTF
, 1, 10, 100 ng/ml HB-EGF가 ,
HB-EGF 2- 72

3.

72
(A),
(B), (C), (D), (E)

0.1% glutaraldehyde (Sigma) 24 1 μg/ml bis-benzimide
trihydrochloride (Hoechst 33342, Sigma) 15
(BX-40, Olympus) , 60 가

4.

0.1% diethyl-pyrocabonate (DEPC, Sigma)가 3
 , TGF- 96
 Ca²⁺, Mg²⁺ phosphate buffered saline (PBS) 1.5 ml micro
 centrifuge tube . TRIzol (Gibco BRL) tube -70
 . Total RNA (reverse transcription) ,
 30 total RNA 가 .
 (polymerase chain reaction) fidelity가
 (Table 1),^{9,19,20} program 94 3 pre-denaturation , 94
 45 denaturation, 54 45 annealing, 72 45 extension
 , 72 10 extension .
 2% agarose gel ethidium-bromide (1 µg/ml, Sigma) 15
 UV light . PCR cDNA
 RNA -actin positive control .
 ATPase -subunit MMP-9 mRNA
 densitometer (Quantity One, BIO-RAD) .

5.

t-test , p<0.05

1. HB-EGF
 2- 1, 10, 100 ng/ml HB-EGF가 가
 , HB-EGF가 가 72
 . 가
 . (A),
 (B), (C), (D), (E)
 . 1 ng/ml 45.1% 38.1%
 가 (p<0.05), 10 ng/ml
 15.1% 10.3% 가 (p<0.05).
 (Table 2).
 , 1
 ng/ml 57.9% 48.3% 가 (p<0.001).
 가 , 10 ng/ml
 HB-EGF가 가
 (Table 3).

2. 2- 72
 . 0.1% glutaraldehyde 24
 trihydrochloride (Hoechst 33342) 15
 가
 가 1 ng/ml 가 91.7±9.2
 82.5±14.6 가 ,

3. MMP-9 ATPase -subunit mRNA HB-EGF
 remodeling MMP-9
 ATPase -subunit HB-EGF가
 internal control -actin mRNA .
 MMP-9 .
 ATPase -subunit mRNA HB-EGF
 가 1 ng/ml
 가 .

growth factor cytokine Vero cell,
^{10,11,12}

Leukemia inhibitory factor (LIF), insulin-like growth factor (IGF), epidermal growth factor (EGF), transforming growth factor (TGF- β), platelet-derived growth factor (PDGF), interleukin-6 (IL-6)
^{13,14,15}

EGF heparin-binding epidermal growth factor (HB-EGF)
^{1,2} 1, 10, 100
 ng/ml HB-EGF 가 2- 72
 가 , 1
 ng/ml (Table. 3).
 1 ng/ml 가 (82.5 \pm 14.6 vs 91.7 \pm 9.2)
 (Table. 4). 가
 가 가
 HB-EGF down-regulation
 desensitization ¹⁶
 paracrine growth factor autocrine
 metalloproteinase LIF EGF , matrix
 factor가 cAMP 가 sodium TGF- β EGF growth
^{16,17,18} HB-EGF MMP-9
 ATPase α -subunit mRNA
 1, 10, 100 ng/ml 72
 RT-PCR MMP-9 가 , ATPase
 α -subunit 1 ng/ml 가 (Figure. 1).
 MMP-9
 growth factor ,
 가 . ATPase
 α -subunit trophectoderm
 1 ng/ml HB-EGF inner cell mass
 trophectodermal cell 가 ATPase α -subunit 가
 HB-EGF cell differentiation
 proliferation 가 ATPase α -subunit 가
 growth factor
 cytokine ,

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Table 1. Primers used for RT-PCR and the size of their amplification products.

Gene		Primer sequence	Position on cDNA	Fragment size (bp)	Reference
-actin	5'	GTGGGCCGCTCTAGGCACCAA	24-45	539	Rappolee et al., 1992
	3'	CTCTTTGATGTCACGCACGATTTTC	541-564		
MMP-9	5'	AGGCCTCTACAGAGTCTTTG	1581-1600	824	Mark et al., 1996
	3'	CAGTCCAACAAGAAAGGACG	2386-2405		
ATPase -subunit	5'	CCCTTCGAGTACGACTATGA	28-47	134	Jones et al., 1997
	3'	TTGACCTGCCTATGTTTCTT	142-161		

Table 2. Effects of heparin-binding epidermal growth factor (HB-EGF) on cavity formation and hatching of mouse embryos which were treated at late 2-cell stage

Treatment	No. of embryos	No.(%) of embryos (post hCG 120hrs)				
		A	B	C	D	E
Control	198	3 (2.5)	25 (12.0)	55 (28.3)	71 (38.1)	21 (10.3)
1 ng/ml	211	2 (1.5)	32 (12.0)	53 (22.9)	85 [*] (45.1)	25 (12.8)
10 ng/ml	210	0 (0.0)	34 (15.0)	62 (28.7)	77 (37.3)	27 [*] (15.1)
100 ng/ml	206	2 (0.7)	38 (17.1)	57 (26.4)	75 (37.0)	20 (12.2)

Degenerated embryos are not shown in this table.

Abbreviation : A, Cavity less than half volume ; B, Cavity greater than half volume ; C, Fully expanded ; D, Hatching blastocyst ; E, Hatched blastocyst

^{*} : Significantly different from control (p<0.05)

Values are obtained from five different experiments.

Table 3. The rate of blastocyst formation and hatching of mouse embryos cultured in medium (HTF) alone and in the presence of 1, 10 and 100 ng/ml HB-EGF

	Post hCG 120hrs			
	HTF	HTF + HB-EGF(1 ng/ml)	HTF + HB-EGF(10 ng/ml)	HTF + HB-EGF(100 ng/ml)
% Blastocyst	91.0	94.7	94.6	93.7
% Blastocyst hatching	48.3	57.9*	42.3	49.2

Abbreviation : HTF, Human tubal fluid ; HB-EGF, heparin-binding epidermal growth factor.

* : Significantly different from the control (p<0.001)

Values are obtained from five different experiments.

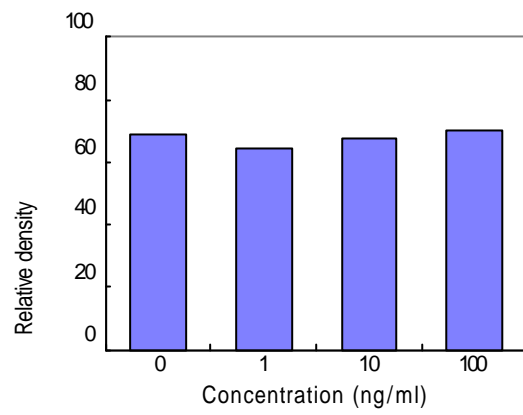
Table 4. Cell number of blastocyst cultured in medium alone and in the presence of 1, 10 and 100 ng/ml HB-EGF

Treatment	Mean cell number
Control	82.5 ±14.6
1 ng/ml	91.7 ±9.2
10 ng/ml	82.3 ±10.5
100 ng/ml	89.4 ±20.9

Cell counts performed at 120 hr post hCG using Hoechst staining.

Embryos with <60 cells excluded from calculation of mean cell number.

A



B

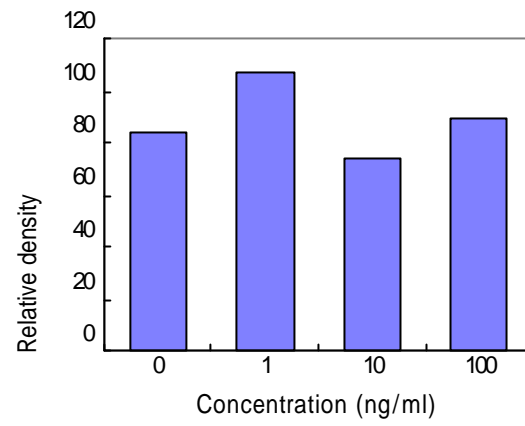


Figure 1. Analysis of MMP-9 (A) and ATPase α -subunit (B) mRNA expression level in the mouse embryos treated with TGF- β . M, 100bp ladder; Lane 1-4, β -actin (539bp); Lane 5-8, MMP-9 (824bp); ATPase α -subunit (134bp).