

배아줄기세포의 인슐린 분비세포로의 유도 분화에 대한 연구

성균관대학교 의과대학 삼성제일병원 생식생물학 및 불임연구실¹, 산부인과²
미즈메디병원 의과학연구소³

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Induced Differentiation of Embryonic Stem Cells to Insulin Secreting Cells

Ji Hye Sung¹, Chun Kyu Lim¹, Hye Won Choi¹, Hyoung Song Lee¹, Hyeonsang Shin¹,
Jin Hyun Jun^{1*}, Hyun Soo Yoon³, Mi Kyoung Koong²

¹Laboratory of Reproductive Biology & Infertility ²Department of Ob/Gyn
Samsung Cheil Hospital, Sungkyunkwan University School of Medicine, Seoul, Korea
³MizMedi Hospital, Medical Research Center, Seoul, Korea

Objective: Embryonic stem (ES) cells could be differentiated into the specific cell types by alternation of culture condition and modification of gene expression. This study was performed to evaluate the differentiation protocol for mouse and human ES cells to insulin secreting cells.

Methods: Undifferentiated mouse (JH-1) and human (Miz-hES1) ES cells were cultured on STO feeder layer, and embryoid bodies (EBs) were formed by suspension culture. For the differentiation, EBs were cultured by sequential system with three stage protocol. The differentiating ES cells were collected and marker gene expressions were analyzed by semi-quantitative RT-PCR in each stage. Amount of secreted insulin levels in culture media of human ES cells were measured by human insulin specific RIA kit.

Results: During the differentiation process of human ES cells, GATA-4, α -fetoprotein, glucose transporter-2 and Ngn-3 expression were increased whereas Oct-4 was decreased progressively. Insulin and albumin mRNAs were expressed from stage II in mouse ES cells and from stage III in human ES cells. We detected 3.0~7.9 μ U/ml secretion of insulin from differentiated human ES cells by in vitro culture for 36 days.

Conclusion: The sequential culture system could induce the differentiation of mouse and human ES cells into insulin secreting cells. This is the first report of differentiation of human ES cells into insulin secreting cells by in vitro culture with serum and insulin free medium.

Key Words: Embryonic stem cell, Insulin secreting cell, Differentiation, Sequential system, Serum and insulin free medium

(1981)

Evans Kaufman

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Tel: (02) 2000-7590, Fax: (02) 2265-5621, e-mail: junjh55@hanmail.net

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(SC12022)

and embryo transfer) (in vitro fertilization) 가 , Assady (2001)
 Thomson (1998) β- 가 ^{10,11}
 (human embryonic stem cell line)가
 , Shamlott (1998)
 (human embryonic germ cell line)
^{2,3} 가 ^{4,5} ¹²⁻¹⁷ 가
 , myelin 가
 dopaminergic
 가 ⁶⁻⁹ 가
 5~10% 가 2000 1.
 1300 , 2005 3000 가
 (insulin-dependent type, IDDM, type 1)
 (non-insulin-dependent type, NI- Lumelsky Moritoh
 DDM, type 2) Type 1 ^{10,16}
 , (JH-1) ,
 β- 가 가 (embryoid
 body) insulin, transferrin,
 diabetic ketoacidosis, hypo- hyper- sodium selenate, fibronectin 가 ITSFn
 glycemia , nephropathy, neuropathy, 6 (stage-I), nicotinamide, in-
 retinopathy, cardiovascular disease sulin, EGF, FGF 가 N2 8
 (stage-II). N2
 가 , B27 supplement 가 22
 (stage-III).
 islet cell , Table 1
 가 RT-PCR
 ,
 2. 가
 Lumelsky (2001)

Table 1. List of marker genes for differentiation of mouse embryonic stem cells

Genes	Sequences	Product sizes
Insulin 1	5'-ccagctataatcagagacca-3' 5'-gtgtagaagaagccacgct-3'	197 bp
Insulin 2	5'-tccgctacaatcaaaaacat-3' 5'-gctgggtagtggtgggtcta-3'	411 bp
Glucagon	5'-actcacagggcacattcacc-3' 5'-ccagtgatgaagtcctcg-3'	353 bp
Somatostatin	5'-tcgctgctgctgaggacct-3' 5'-gccagaagtactggccagttc-3'	232 bp
IAPP	5'-actagctcagcacacaggat-3' 5'-agacaagagaggctgcaagt-3'	364 bp
Pdx-1	5'-accatgaacagtgaggagca-3' 5'-tcctctgttttctcgggt-3'	451 bp
Pax-4	5'-aatggcgagcagcaagagaa-3' 5'-atgaggaggaagccacagga-3'	280 bp
Isl-1	5'-agatatgggagacatggcgat-3' 5'-acacagcggaaacactcgatg-3'	327 bp
Ngn-3	5'-tggcactcagcaaacagcga-3' 5'-accagagccagacaggtct-3'	444 bp
Beta-2	5'-cttggccaagaactacatctgg-3' 5'-ggagtagggatgaccgggaa-3'	222 bp
Nkx2.2	5'-aacctgcccagcgcgctcaa-3' 5'-agggcctaaggcctccagtct-3'	220 bp
Glucose transporter-2	5'-cgggtaggactgtgctgctgg-3' 5'-ctctgaagacgccaggaattccat-3'	416 bp
Glucokinase	5'-tggatgacagagccaggatgg-3' 5'-acttctgagccttctggggtg-3'	208 bp
Kir6.2	5'-ggctcctagtacgtgacca-3' 5'-ccacagccactgcgcttgcg-3'	317 bp
PC2	5'-agagattcattgtgtggga-3' 5'-caaatggacttggtgcca-3'	215 bp
Oct-4	5'-acctccccatggc-3' 5'-acttgatctttggccttctg-3'	855 bp
GAPDH	5'-accacagtcctgcatcac-3' 5'-tccaccacctgtgctgta-3'	452 bp
β-actin	5'-gtatgctctggtctgacca-3' 5'-cttctgcatctgtcagca-3'	499 bp

Table 2. List of marker genes for differentiation of human embryonic stem cells

Genes	Sequences	Product sizes
Insulin	5'-gcctttgtgaaccaacacctg-3' 5'-gttcagtagttctccagctg-3'	261 bp
Pdx-1	5'-eccatggatgaagtctacc-3' 5'-gtcctcctctttttccac-3'	262 bp
-fetoprotein	5'-tgaaaacctcttgaatgcc-3' 5'-tcttgcttcatcgttgacg-3'	492 bp
Glucose transporter-2	5'-aaccagcattttcagacgg-3' 5'-agcactccagcaagaggaa-3'	441 bp
Sox-17	5'-agtgacgaccagagccagac-3' 5'-ccttagccccacacatgaaa-3'	214 bp
Ngn-3	5'-cccttactctcccagctcc-3' 5'-ccttaccttagaccacca-3'	176 bp
GATA-4	5'-gacgggtcactatctgtgcaac-3' 5'-agacatgcactgactgagaac-3'	475 bp
Somatostatin	5'-cccagactccgtcagttct-3' 5'-ccatagccgggttgagttta-3'	205 bp
Glucagon	5'-ctcagtgatcctgatgatgaac-3' 5'-agtccctggcggcaagattatcaag-3'	370 bp
Oct-4	5'-cgtgaagctggagaaggagaagctg-3' 5'-aaggcccagcttacacatgttc-3'	244 bp
GAPDH	5'-agccacatcgctcagacacc-3' 5'-gtactcagcggcagcatcg-3'	302 bp
β-actin	5'-tggcaccacaccttacaatgagc-3' 5'-gcacagcttctcctaatgtcacgc-3'	296 bp

body) ITSFn
6 (stage-I), N2 insulin
IGF-I IGF-II 가 N2-insulin free
8 (stage-II).
N2, N2+B27 N2-insulin free, N2+
B27-insulin free 22
(stage-III).
, Table 2 RT-POR

3. RIA

(Miz-hESC1)

(embryoid stage-III)

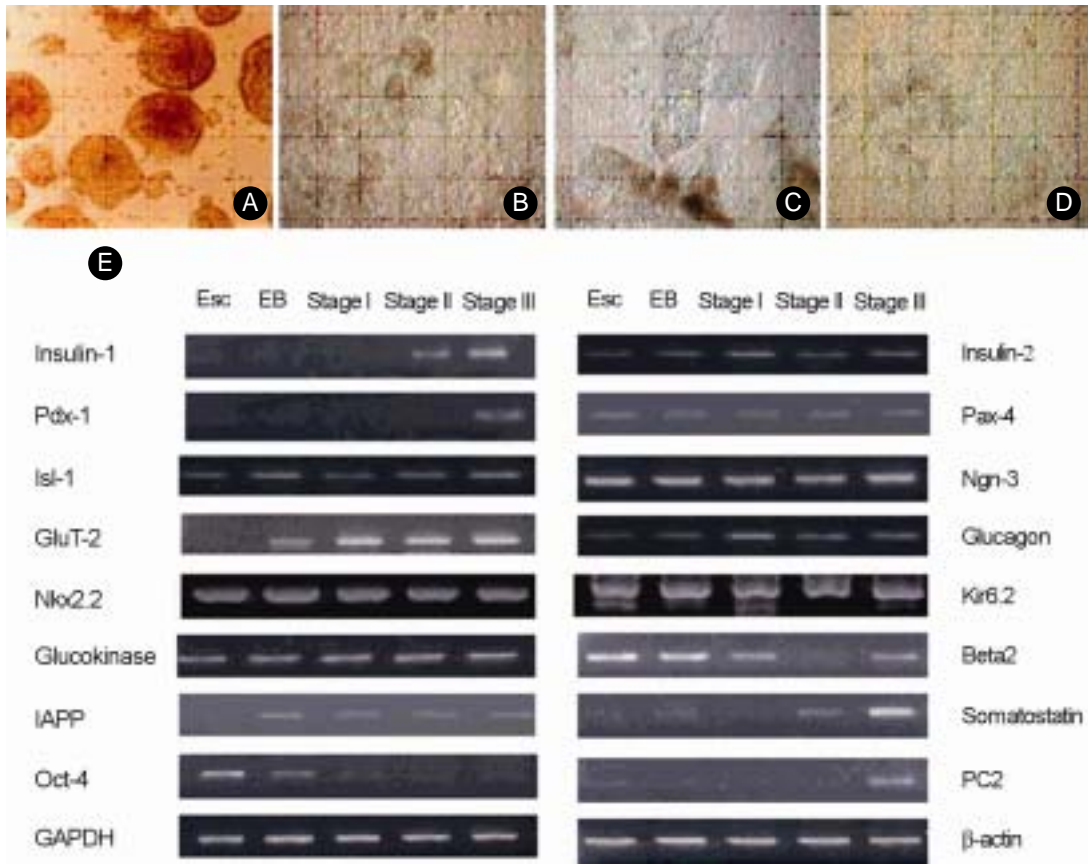


Figure 1. Morphological observation (A~D) and RT-PCR analysis (E) during the differentiation of mouse embryonic stem cells. **A:** EB stage, **B:** Stage-I (ITSFn for 6 days), **C:** Stage-II (N2 for 8 days), **D:** Stage-III (N2 for 22 days), **E:** Gel electrophoresis of RT-PCR products by sequential culture system. GAPDH and β -actin gene were used as internal controls.

RIA kit human insulin μ U/ml
 (Linco Research Inc., USA) Kruskal-Wallis
 0.1% bovine serum albumin (BSA) 5 mM ANOVA test Dunn's multiple comparison test
 glucose 가 가 Krebs buffer 10 p 0.05
 , glucose
 5 mM 25 mM glucose가 Krebs buffer
 3
 RIA kit
 insulin , 1.
 가 bovine insulin
 (<0.1 μ U/ml) Standard
 quality control sample radio
 activity gamma counter au-
 tomated data reduction procedure (Figure 1 A-D),

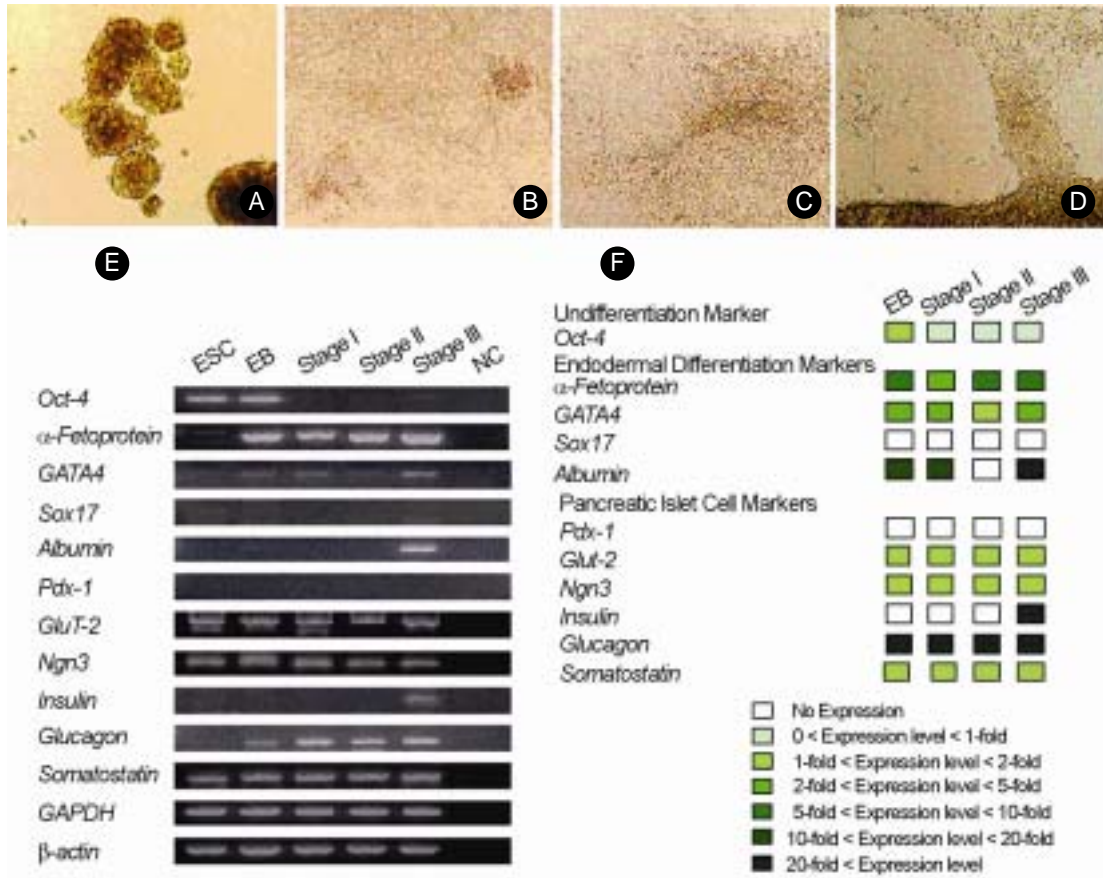


Figure 2. Morphological observation (A~D) and RT-PCR analysis (E, F) during the differentiation of human embryonic stem cells. **A:** EB stage, **B:** Stage-I (ITSFn for 6 days), **C:** Stage-II (N2 for 8 days), **D:** Stage-III (N2 for 22 days). **E:** Gel electrophoresis of RT-PCR products by sequential culture system. **F:** Schematic interpretation of the expression pattern of marker genes. Data were calculated by the expression ratio of each group to undifferentiated human embryonic stem cells.

RT-PCR (Figure 1 E). Oct-4

Oct-4 GATA-4, alpha fetoprotein, glucose transporter-2, Ngn-3

가 가 가 , albumin insulin mRNA

, insulin-1, Pdx-1, somatostatin PC2 stage-III

stage-III 가 (Figure 2 E).

2. semi-quantitative RT-PCR (Figure 2 F).

3.

(Figure 2 A-D). (stage-III)

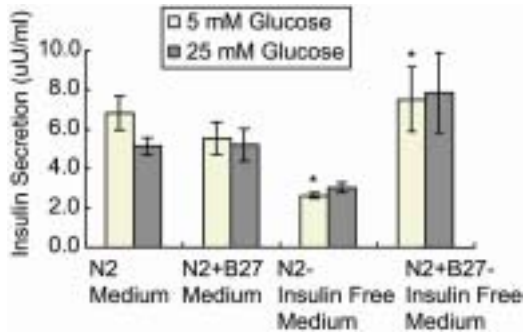


Figure 3. Amount of secreted insulin from differentiated human embryonic stem cells (stage-III). *p<0.05.

Figure 3
 3.0~7.9 µU/ml
 N2
 free
 µU/ml
 µU/ml
 (p<0.05).
 N2 + B27
 5 mM glucose
 N2+B27-insulin free
 N2-insulin
 3.0 ± 0.2
 7.9 ± 2.0
 glucose

가 glucose¹⁸
 glucose
 Pax-4
 가 가¹⁴, Moritoh
 (2003) Pdx-1

oter¹⁶ Nkx6.1 prom-
 ent (embryonic day 17.5) pancreatic rudim-
 가¹⁹,
 cell clusters
²⁰
 β-

가
 insulin
 가

가
 가

가
 glucose
 β-
 가
 Sipione (2004)

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