

12-o-tetradecanoyl-phorbol-13-acetate가 -hCG



=ABSTRACT=

Effects of 12-o-tetradecanoyl-phorbol-13-acetate on -hCG secretion by cultured peripheral mononuclear cells during pregnancy

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Objective : Peripheral blood mononuclear cells (PBMC) in culture release a biologically active human chorionic gonadotropin (hCG). This effect is detectable during pregnancy with a maximum between the 16th and 19th week. HCG plays an important role for the corpus luteum rescue during the early gestational age and possibly for the immunotolerance. This study was performed to investigate the relationships between the productivity of cultured PBMC of pregnant women and the ability to maintain early pregnancy, and whether 12-o-tetradecanoyl-phorbol-13-acetate (TPA) increases hCG secretion by cultured PBMCs.

Materials and Methods : PBMC were obtained from 20 pregnant women between 16th to 19th week of gestation, and cultured with TPA. Culture cells were harvested and hCG mRNA were extracted and RT-PCR were performed. Culture supernatants were collected and hCG concentration were determined by commercial RIA methods.

Results : The mean age was 31.0 years old, 19 of 20 (95%) pregnant women's PBMC secreted hCG and expressed hCG mRNA, but in control group except male hepatitis B patient, none of them produced hCG. TPA activated expression of hCG in PBMC in linear manner.

Conclusion : Pregnant women's cultured PBMC secreted hCG, but not in non-pregnant or male. We could confirm the mRNA of hCG in PBMC as well in the placental control. The productivity of hCG in PBMC might be closely related with maintenance of early pregnancy.

Key words : PBMC, pregnancy, TPA, hCG

Human Chorionic Gonadotropin (hCG)	!	hCG가
(trophoblast cells)		가
(corpus luteum)	(deciduali-	hCG
zation)	(progesterone)	, ^{2,3,4,5} -hCG가
	IL-2	sIL-2R

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6 hCG가 가 400 × g, 15 30
(peripheral blood mononuclear cell, PBMC)
(hydatidiform mole) (choriocarcinoma) PBMC HBSS 3
(trophoblastic disease), hCG
(germ cell tumor) trypan blue dye exclusion test
hCG^{7,8,9} 10% FBS, 250 U/ml penicillin, 250 µg/ml
(tumor marker)^{10,11} streptomycin 가 RPMI-1640 1 ×
hCG가¹² 107 /ml , 96 well culture plate
hCG가 250µl 5% CO₂, 37
¹³ 40 ng/ml TPA 가
1986 Harbour-MacMenamin,¹⁴ 1993 Alexander¹⁵ , 12 24 well 250µl
(peripheral blood mononuclear cell, PBMC) radioimmunoassay -20
hCG
1998 Alexander¹⁶ hCG mRNA
(Natural killer cell, NK cell) 2) (RT-PCR)
(monocyte) hCG가 , IL-1 RNA RNAzol
, IL-4, IL-6, IL-10, TNF GM-CSF hCG , RNA RNAzol
IL-2 INF- hCG 1ml RNAzol
hCG 150µl chloroform 15
가 hCG 4 , 8,000 rpm 15
isopropanol 가 4 , 8,000 rpm 15
가 ,
hCG 가 1ml 75% ethanol 가 4 , 8000
RT-PCR -hCG mRNA rpm 8
, TPA가 hCG 100µl 1mM EDTA 60 10
RNA Ultraspec 3000
UV/Visible spectrophotometer [Pharmacia] RNA
RNA 1
µg/µl
(Reverse transcription) 2.5µl
1. RNA 70 10 가
2000 1 2000 3
16
19 20 .
µl 10 mM dNTP mixture, 15 U AMV Reverse
Transcriptase, 1µl Oligo(dT)15 Primer, 0.5µl
Recombinant RNasin Ribonuclease Inhibitor
Nuclease-Free Water
42 15
99 5 가 0
2. 15 가 cDNA
1) -20
2.5Ml
Hank's Balanced Salt Solution
(HBSS, pH 7.2) 가 , 4Ml Ficoll-Paque
[Pharmacia] 15ml conical centrifuge pasteur pipette
PCR Bioneer PreMixTM-Top
Perkin-Elmer GeneAmp PCR System 9700
PCR 10mM Tris HCl,
pH 8.3, 50mM KCl, 1.5mM MgCl₂, 0.2 mM each dNTP,