O-8 Cellular Modification of Human Embryonic Stem Cells by Pdx1 Protein Transduction

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Background & Objectives: Many strategies for genetic modification, such as plasmid transfection, homologous recombination, and virus transduction, have not been applied efficiently to hESC and this is a major limitation in hESC-involved research. To overcome this limitation, we applied protein transduction technology which can transfer proteins into cells via direct penetration across the lipid bilayer. In this study, we investigated that whether TAT protein transduction domain (PTD)-mediated transduction works in hESC and the transduced protein functions inside hESC.

Method: The human embryonic stem cell line, SNUhES3 (passage 30~50) was used in this study. hESC was treated with TAT-FITC peptide that contains TAT PTD (a stretch of 11 amino acids from HIV-1 TAT protein) and FITC fluorescent dye and then peptide transfer was analyzed using confocal microscopy and flow cytometry. The full-length cDNA of Pdx1 which plays a central role in regulating pancreatic development and insulin gene transcription was subcloned into pET21b vector containing TAT PTD sequence. TAT-Pdx1 fusion protein was expressed in E. coli BL21 (DE3) and purified using His-tag affinity chromatography. Day 1 embryoid bodies (EB) was treated with TAT-Pdx1 and transduction was confirmed by western blotting. To investigate that the transduced TAT-Pdx1 protein is functional inside hESC, the expression of downstream target genes of Pdx1, insulin, somatostatin, glucokinase, GLUT2, and pdx1 itself, was monitored by quantitative RT-PCR. In addition, insulin protein expression was analyzed by immunocytochemistry.

Results: After hESC was treated with TAT-FITC at a concentration of 5 uM for 1 hr, most of cells showed green fluorescence by confocal microscopy. In case of day 1 EB, same result was obtained and flow cytometry analysis revealed more than 70% of cells had fluorescence. Day 1 EB was treated with TAT-Pdx1 protein at a concentration of 5 ug/ml and then transduction kinetics was monitored by western blotting. After treatment of 30 min, TAT-Pdx1 was detected and protein level was sustained for more than 24 hrs. These results demonstrate rapid transduction process in hESC and high stability of the protein in culture medium. To investigate that the transduced TAT-Pdx1 protein is functional inside hESC, Day 7 EB was treated TAT-Pdx1 for a week and quantitative RT-PCR against downstream target genes of Pdx1 was performed. The result showed increase in insulin and pdx1 mRNA levels. Also, the result of immunocytochemistry using insulin antibody confirmed that there was actual increase in insulin protein level.

Conclusions: In this study, we showed high efficiency of protein transduction in hESC and this is first report in the field of embryonic stem cells. In addition, we demonstrated that the transduced TAT-Pdx1 was functional inside hESC and generated insulin-producing cells from hESC. Therefore, protein transduction technology could be used for a variety of differentiation-inducing proteins and applied to cell-based therapies via induced differentiation of hESC into particular cell types and analysis of gene function involved in

human embryogenesis.

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O-9 백서의 배란과정중 Pituitary Adenylate Cyclase-Activating Polypeptide의 작용

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Background & Objectives: 배경: Pituitary adenylate cyclase-activating polypeptide (PACAP)은 양의 시상하부에서 추출된 신경펩타이드 호르몬으로 난소에도 존재하여 배양된 과립막 세포에서 스테로이드합성과 cyclic AMP 대사를 촉진함이 보고되었다. 목적: 흰쥐 난소를 실험 모델로 사용하여 배란시 황체화호르몬 (luteinizing hormone; LH)에 의해 유도된 PACAP과 PACAP수용체의 유전자 발현양상과 신호전달경로를 규명하고자 시도되었다.

Method: 미성숙 흰쥐의 배란전 난포를 체외 배양하면서 LH로 처리하고 PACAP 및 PACAP수용체의 유전자 발현을 보기 위해서는 Northern blot 분석과 in situ hybridization (ISH)을, 그리고 단백질 수준의 PACAP 검색을 위해서는 enzyme linked immunosorbent assay (ELISA)분석을 이용하였다.

Results: LH처리후 Northern blot상의 PACAP 유전자 발현은 6~9시간에 일시적으로 최고치에 도달하였으며 ISH로 보아 과립막 세포에서 발현됨을 알 수 있었다. ELISA 분석 상 PACAP 단백질도 LH처리 후 6~12시간에 최고치를 나타내었으며, PACAP수용체 mRNA 역시 3~9시간에 최고치로 과립막 세포에서 발현되었다. Adenylate cyclase (AC) 억제제인 MDL12330A처리시 LH로 발현된 PACAP mRNA가 감소되며, AC의 활성제인 forskolin처리에는 LH시와 유사한 PACAP mRNA의 발현 양상을 나타내었다. 그러나 protein kinase C (PKC)의 억제제인 chelerythrine과 2-0-tetradecanolphorbol-13-acetate (TPA)처리로는 PACAP의 유전자 발현에 영향을 주지 못하였다. 5-lipoxygenase의 억제제인 MK886이나 nordihydroguaiaretic acid (NDGA)로 처리한 결과 LH로 유도된 PACAP 유전자의 발현이 감소되었으나, cyclooxygenase의 억제제인 indomethacin은 별로 영향을 주지 못하였다. MEK와 p38의 억제제인 PD98059와 SB203580도 LH로 촉진된 PACAP의 유전자 발현을 농도 의존적으로 억제하였다.

Conclusions: 배란전 난포에서 PACAP과 PACAP수용체의 유전자 발현은 모두 LH의 폭발적 분비에 의해 유도되어 일시적으로 과립막 세포에서 나타나 배란을 위한 국소적인 조절 작용을 할 것으로 추정되며, LH로 촉진된 PACAP 유전자 발현을 위한 신호전달은 cAMP-PKA, lipoxygenase 및 MAP kinase 경로를 통하는 것으로 사료된다.