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Differentiation to kidney precursor cells from mouse embryonic stem cells

*Won KIM, Tung NGUYEN-THANH, Yu jin JUNG, Woong PARK , Kyung pyo KANG, Myung kwan HAN

Internal Medicine, Chonbuk National University Medical School, Korea, South

Objectives : Embryonic stem (ES) cells have the capacity to differentiate into all cells of the developing embryo and may provide a renewable resource for future cell replacement therapies. This study was to evaluate a protocol to differentiation of mouse embryonic stem cells toward kidney precursor cells via embryoid bodies (EB).

Methods : Mouse embryonic stem cell, R1 cells were maintain on feeder layers of mitomycin C-treated mouse embryonic fibroblasts in cultivation medium consisting of DMEM supplemented with 15% ES Cell Qualified FBS, GlutaMAX Supplement, β -mercaptoethanol, non-essential amino acids and leukemia inhibitory factor (LIF). To induce renal lineage, aliquots of 20 μ l media without LIF containing 1000 cells were cultivated in "hanging drops" for 2 days. The EBs were plated onto gelatin coated 6 cm tissue culture plates. Differentiation was performed in DMEM medium, 2% ES-FBS, GlutaMAX Supplement, β -mercaptoethanol, non-essential amino acids with 100 nmol/L retinoic acid and 10 ng/mL activin A. The plated EBs were cultivated for up to 21 d after plating. Gene expression of kidney progenitor cells (CITED1, Six2 and WT1), intermediate mesoderm (Pax2, Lhx1), and pre-tubular aggregate (Pax8) markers were performed by quantitative real-time RT-PCR.

Results : Our data demonstrate that marker molecules characteristic for kidney progenitor (CITED1, Six2 and WT1) were found to be dramatically increased expression during EB cultivation. CITED1 gene expression reach a peak at day 16 with more than 88-fold changes (88.1 ± 33.2). The peak gene expression of Six2 is earlier at day 9 (475.2 ± 219 fold changes). Meanwhile WT gene expression dramatically increase at day 2 (34.8 ± 11.3 fold changes) and then decrease during differentiation period. Intermediate mesoderm (Pax2, Lhx1) also future increases after culture EB on retinoic acid and activin A media. At day 16, Lhx1 mRNA expression reach more than 4500-fold changes ($4,591.1 \pm 1820.1$), Pax2 reach 44.2 ± 28.8 fold changes. However, Pax8, marker molecules involved in Pre-tubular aggregate were not significantly change.

Conclusions : Differentiation of mouse embryonic stem (ES) cells via embryoid bodies (EB) toward kidney precursor cell is established as a suitable protocol to

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study cellular processes of renal development in vitro.

Keywords : mouse embryonic stem, embryoid bodies, kidney precursor, intermediate mesoderm, pre-tubular aggregate