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Differentiation of Human ES Cells to Endodermal Lineage Cells

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Embryonic stem (ES) cells have property of self-renewal and can differentiate into the cells of all three primary germ layers. Recently, many growth factors, alteration of culture condition and gene modifications have been used to differentiate mouse and human ES cells into specific cell types. This study was performed to evaluate the differentiation protocol for human ES cells to the endodermal lineage cells. Human ES cells (Miz-hES1) were cultured on STO feeder layer mitotically inactivated with mitomycin C, and embryoid bodies (EBs) were formed by suspension culture. Differentiation protocol of EBs consisted of three steps: stage I, culture of EBs for 6 days with ITSFn medium; stage II, culture of stage I cells for 8 days with N2 medium; stage III, culture of stage II cells for 22 days with N2 medium. mRNA levels of the endodermal lineage differentiation genes were analyzed by semi- quantitative RT-PCR. The *Oct-4* expression, a marker of the pluripotent state, was detected in undifferentiated human ES cells but progressively decreased after EBs formation. Differentiating human ES cells expressed marker genes of endodermal differentiation and pancreatic islet cells. *GATA4*, *α-fetoprotein*, *Glut-2*, and *Ngn3* were expressed in all stages. However, albumin and insulin were expressed in only stage III cells. The human ES cells can be differentiated into endodermal lineage cells by multiple step culture system using various supplements. We are developing the more effective protocols for guided differentiation of human ES cells.

Key words) ***human ES cells, endodermal cells, differentiation, RT-PCR***