Available online at www.sciencedirect.com
ScienceDirect
Diabetes Research and Clinical Practice 77S (2007) S138–S142
DIABETES RESEARCH AND CLINICAL PRACTICE
www.elsevier.com/locate/diabres

Both Pdx-1 and NeuroD1 genes are requisite for the maintenance of insulin gene expression in ES-derived differentiated cells

Koichi Saitoh, Eiji Yamato *, Satsuki Miyazaki, Jun-Ichi Miyazaki

Division of Stem Cell Regulation Research, Osaka University Graduate School of Medicine, Osaka, Japan

Accepted 29 January 2007
Available online 23 April 2007

Abstract

Embryonic stem (ES) cells can differentiate into many cell types. Recent reports have shown that ES cells can differentiate into insulin-producing cells. We have established an ES cell line in which exogenous Pdx-1 expression was precisely regulated by the Tet-off system integrated into the ROSA26 locus and succeeded to produce insulin-producing cells. The Pdx-1 expressing final differentiated insulin-positive cells can be maintained for more than 2 months. However, in spite of their induced expression of Pdx-1, the repeated passages of cells lost their capacity to express insulin and NeuroD1 gene. Forced expression of NeuroD1 gene by adenoviral vector in these cells restored the expression of insulin. These results suggested that maintenance of the property of insulin-producing cells derived from ES cells could be achieved by synergistic expression of Pdx-1 and NeuroD1.

Keywords: ES cell; Pdx-1; NeuroD1; Insulin

1. Introduction

Embryonic stem (ES) cells are derived from the inner cell mass of blastocyst-stage embryos and contribute to all adult tissues, including germ cells, when reintroduced into host blastocysts. To date, ES cells have been also shown to differentiate in vitro into various cell lineages including those of hematopoietic precursors, heart muscles, skeletal muscles, endothelium, and neural cells. Thus, differentiation events similar to those occurring in vivo can be attained in cell culture using ES cells.

The differentiation of ES cells into lineage-restricted cells is expected to be a promising technique for future therapeutic use, including diabetes treatment. Lumelsky et al. developed a five-stage method, including a selection step for nestin-positive cells, for the differentiation of mouse ES cells into insulin-secreting cells [1]. We have also reported the differentiation of insulin-producing cells from a feeder-free ES cell line harboring the β-geo gene under the control of the mouse insulin 2 promoter by a method similar to the five-stage method. However, the differentiation is not efficient enough to produce insulin-secreting cells.

Pdx-1 is homologous to a Xenopus endoderm-specific homeodomain protein, XIHbox8. In fact, pdx-1 transactivates insulin gene expression through conserved enhancer elements, and is also an essential regulator of pancreatic development. However we noted that the levels of Pdx-1 expression were very low throughout the

* Corresponding author at: Division of Stem Cell Regulation Research, G6, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan. Tel.: +81 6 6879 3824; fax: +81 6 6879 3829.
E-mail address: yamato@nutri.med.osaka-u.ac.jp (E. Yamato).

0168-8227/$ – see front matter
doi:10.1016/j.diabres.2007.01.048

differentiation stages of ES cells [2]. Thus, we expected the strategy of leading the ES cells to express the transcription factor Pdx-1 during in vitro differentiation would improve the efficiency of producing cells of the pancreatic lineage, and especially β cells. Based on these considerations, we attempted to inducibly express exogenous Pdx-1 during the in vitro differentiation of ES cells and showed that Pdx-1 expression clearly enhanced the expression of the insulin 2, somatostatin, Kir6.2, glucokinase, neurogenin3, p48, Pax6, PC2, and HNF6 genes in the resulting differentiated cells [3].

We have also proved that those final differentiated cells (ROSA-PDX cell cluster) can be cultured for several months in the previous report [3]. Although preservative cells are important as a candidate for the source of regenerative insulin-producing cells, there are no data for the effect of long-term culture of differentiated ES-derived insulin-producing cells. Thus, in this study, the effect of long-term culture of ROSA-PDX cell cluster was investigated.

2. Materials and methods

2.1. ES cell culture

The ES cells, which we have produced, express exogenous Pdx-1, precisely regulated by the Tet-off system integrated into the ROSA26 locus. The ES clones constitutively and homogeneously expressed tTA under the ROSA26 promoter. The tTA protein activated the downstream CMV*1 promoter to produce pdx-1 as well as EGFP. Thus we can monitor exogenous Pdx-1 gene expression by EGFP expression (Fig. 1a).

The ES cells were subjected to an in vitro differentiation procedure basically as described previously [3]. Before the induction of differentiation, the ES cells were cultured in the presence of Dox. The cells were dissociated with 0.25% trypsin/0.04% EDTA in PBS and plated onto bacterial culture dishes in DMEM containing 10% FCS in the presence Dox. Embryoid bodies (EBs) then formed within 4–5 days. The EBs were replated onto gelatinized plates in DMEM containing 10% FCS. The medium was replaced with serum-free ITSFn medium, and the cells were cultured for 3–6 days. The surviving cells were dissociated and replated onto gelatinized plates in MHM medium; that is, DMEM/F12 (1:1) medium containing 25 μg/ml insulin, 100 μg/ml transferrin, 20 nM progesterone, 60 μM putrescine, 30 nM sodium selenite, 10 ng/ml human keratinocyte growth factor (KGF, Peprotech EC, London, UK), 20 ng/ml epidermal growth factor (EGF, Sigma–Aldrich), 25 ng/ml basic fibroblast growth factor (bFGF, Strathmann Biotech GmbH, Germany), B27 supplement (Invitrogen), and 10 ng/ml nicotinamide [2], and cultured for 6–8 days. The expanded cells were dissociated and replated onto gelatinized plates in MHM medium without bFGF, EGF, or KGF. These cells could be cultured for more than 2 months (ROSA-PDX cell cluster).

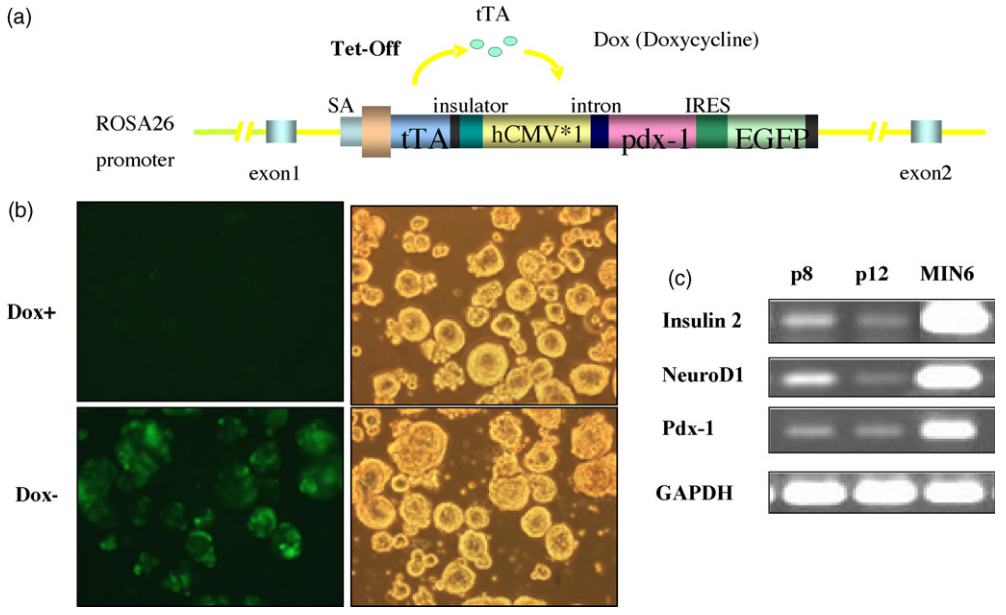

Fig. 1. (a) ES cells, used in this study, confer the Tet-off regulation unit for pdx-1 expression into the ROSA26 locus [3]. In this cell line, withdrawal of tetracycline, doxycycline (Dox), induces the expression of Pdx-1 and EGFP genes. (b) Gene expression in ROSA-PDX cell cluster analyzed by RT-PCR. Withdrawal of Dox induces the expression of EGFP in ROSA-PDX cell cluster. (c) Gene expression pattern of p8 and p12 ROSA-PDX cell cluster. Insulin 2 and NeuroD1 gene expression levels ware decreased in p12 ROSA-PDX cell cluster. In contrast, Pdx-1 gene expression was not changed.