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EFFECT OF DOXYCYCLINE-REGULATED ERP57 EXPRESSION ON THROMBOPOIETIN PRODUCTIVITY IN RECOMBINANT CHO CELLS

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ABSTRACT

To determine the effect of ERp57 expression on thrombopoiein (TPO) productivity in recombinant Chinese hamster ovary (rCHO) cells, TPO producing rCHO cell line with doxycycline-regulated ERp57 expression was developed. The Erp57 expression level could be regulated by addition of different concentrations of doxycycline to culture medium. The doxycycline concentration of 1 μg/ml was high enough to suppress the ERp57 expression. Up to 5μg/ml doxycycline concentration used in culture medium, no observable cytotoxic effect of doxycycline was detected during culture. Overexpression of ERp57 was found to increase the specific TPO productivity (q_{Tpo}) without growth inhibition, probably due to the chaperone-like activity of ERp57 in CHO cells.

INTRODUCTION

Thrombopoietin (TPO) is a recently identified hematopoietic growth factor that induces thrombopoiesis by proliferation of megakaryocyte progenitors of platelets and differentiation of immature megakaryocyte. The lumen of the endoplasmic reticulum (ER) contains a number of molecular chaperones that assist in the later stages of protein biosynthesis and folding. PDI also functions as a molecular chaperone and has been found to be associated with misfolded proteins in the ER. ERp57 is a 57kDa ER protein that is one of the PDI family proteins. ERp57 is also known as a thiol-dependent reductase and putative cysteine protease. A number of studies show specific interactions between newly synthesized glycoproteins and the putative chaperones such as calnexin and calreticulin[1]. Like calnexin and calreticulin, ERp57 also modulates glycoprotein folding[2]. Gossen and Bujard first described tetracycline-controlled gene expression system in 1992[3]. By adding increasing level of tetracycline, a luciferase reporter gene was repressed from high level to basal level. Using this Tet-Off system, we investigates the effect of Erp57 expression level on q_{Tpo} of rCHO cells.

MATERIALS AND METHODS

Cell Line Development. The rCHO cell line expressing TPO (TPO-33) was made by transfection of TPO expression plasmid into DHFR-deficient CHO cells. The TPO-33 cells expressing Tet-Off system (TPO-33-Tet-Off, used as a negative control) were selected by luciferase assay. The TPO cell line overexpressing ERp57 protein (TPO-33-ERp57) was established by co-transfecting TRE-ERp57 expression vector (pTRE-ERp57) with pTK-Hyg vector into TPO-33-Tet-Off cells and selected by 200μg/mL hygromycin.

Culture Maintenance and Culture. The ERp57 overexpressing cells (TPO-33-Erp57) were cultivated as monolayer cultures in 75-cm^2 T-flasks containing 15 mL of IMDM supplemented with 10% dFBS, and 80n*M* MTX and passed every 3-4 day upon reaching confluency.

Western Blot Analysis against ERp57. To confirm the ERp57 overexpression, Western blotting was performed. ERp57 was probed with goat anti-mouse ERp57 polyclonal antibody (StressGen, Canada).

Analytical Methods. Cells concentration was estimated using a hemacytometer. Viable cells were distinguished from dead cells by the trypan blue dye exclusion method.

RESULTS AND DISCUSSION

Vector Construction and Double Stable Cell Line. The rCHO cell line producing TPO was established previously and pTet-Off cell line was developed from the TPO-33 cell line. The ERp57 cDNA fragment was obtained by PCR and inserted into the EcoRI/Xbal site of pTRE vector (CLONETECH). The transcription of ERp57 cDNA sequence is controlled by a minimal CMV promoter, which lacks the enhancer that is part of the complete CMV promoter in the pTet plasmids. The pTRE-ERp57 (Figure 1) plasmids are cotransfected with pTK-Hyg to permit selection of stable transfectants. To select stably transformed cells, 200μg/ml of hygromycin was used.

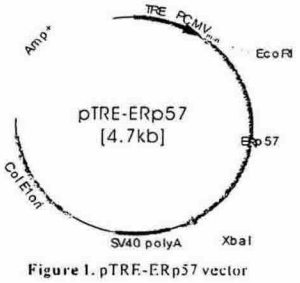

Figure 1. pTRE-ERp57 vector

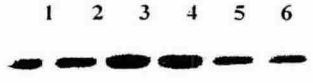

Figure 2. Western blot analysis of doxycycline-regulated expression ERp57. TPO-33-Tet-Off cells were transfected with the pTRE-ERp57 and pTK-Hyg vector and then cultured for 72hr in the presence/absence of the doxycycline. Lane 1, 2 : TPO-33-Tet-Off (as a control), 3, 4 : TPO-33-ERp57 without doxycycline (switch on), 5, 6 : TPO-33-ERp57 with 1μg/ml doxycycline (switch off)

(n=6)	μ (day^{-1})
Control without doxycycline	0.660 ± 0.031
Control with 5μg/mL dox	0.655 ± 0.052
Transfectant without dox	0.650 ± 0.048
Transfectant with 0.01μg/mL dox	0.652 ± 0.045
Transfectant with 0.1μg/mL dox	0.668 ± 0.055
Transfectant with 1μg/mL dox	0.611 ± 0.098
Transfectant with 5μg/mL dox	0.657 ± 0.052

Table 1. Comparison of specific growth rate

Batch Culture of TPO producing rCHO cells with controlled ERp57rexpression. Figure 2 shows overexpression of ERp57 (lane 3, 4) without doxycycline as compared to the negative control (lane 1, 2). After 3 days batch culture, cell lysates were prepared for Western blotting. Cultured cells with 1μg/ml doxycycline (lane 5, 6) showed ERp57 protein at a basal level. Table 1 shows doxycycline has no cytotoxic effect on the growth of rCHO cells. It was found that overexpression of ERp57 increased the specific TPO productivity (q_{Tpo}) without growth inhibition.

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