Transposon-based technology enhances the generation of stable and high-producing CHO clones for industrial production of recombinant hFGF23 protein

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Abstract

In the last 15 years, different approaches for gene transfer into mammalian cells have been developed, however it is still challenging to obtain stable, high-producing cell lines for industrial applications. Conventional methods, based on spontaneous integration of nuclear DNA, often result in low transgene expression due to plasmid concaters silencing and/or position effects. To overcome these limitations we used transposons, as new gene transfer method (Matasci et al., 2011). The PiggyBac (PB) transposon system relies on the ability of transposase to catalyze single transgene integration into actively transcribed regions of genome. Using both PiggyBac transposon technology and conventional methods, we generated stable CHO cell lines expressing soluble and properly folded recombinant human fibroblast growth factor 23 (hFGF23), a regulator of phosphate homeostasis and vitamin D metabolism. Our results demonstrate that PB transposition increases the frequency of stable cell lines generation up to 10 fold compared to nude plasmid transfection. In addition, cell lines establishment is faster and the frequency of high-producing clones is enhanced. Best candidate clones have been adapted to suspension culture and, in batch culture production, they show an average productivity of 20 mg/L of secreted and soluble HGF23 protein. In conclusion, the PB transposon system can be considered a quick, powerful alternative to standard method for generation of stable, high-producing recombinant mammalian cell lines for industrial protein production.

Introduction

Transposable elements (TE) are DNA segments with the ability to move from one genetic location to another. TE falls into two major classes according to whether their replication intermediate is RNA (transposon) or DNA (transposon). DNA transposons move directly as DNA fragment by a conservative (cut-and-paste) mechanism of transposition. They consist of a single gene encoding the transposase enzyme, which is flanked by terminal inverted repeats (TIR). The transposase enzyme recognizes the binding site within TIR and catalyses the DNA fragment excision from donor locus and subsequently its reinsertion elsewhere. PiggyBac (PB) transposon is a TE that specifically targets TATA sites in genome. Recently it has been harnessed for non-viral transgenes delivery into cultured mammalian cells1.

The power of this system is that transposase can mobilize transposons in trans, as long as their retain the TIR. Therefore, in the molecular tool, the transposase gene is physically separated from the TIR and replaced by a gene of interest. The resultant transposon is carried by a plasmid vector, while the transposase is supplied on a helper plasmid. Through the cut and paste mechanism the gene of interest is excised from the donor molecule and in single-copy integrated into the host genome.

Results

Enhancing frequency of high-producing CHO cell lines

To develop hFGF23 high-producing CHO cell lines we cloned different promoters into transposon vector to compare their strength (promt, 2, 3, 4). PB transposon vectors expressing HGF23R179Q sequence under the control of selected promoters were transfected into CHO-K1 cells using a transposon/transposase molar ratio of 2.5:1. As a control experiment, cells were transfected with pCDNA3.1_hGF23R179Q standard vector (Std).

Commercial vs purified hFGF23

The two highest performing clones in serum-free suspension culture were compared in 7 days batch production (Sarstedt-miniPerm and Flask) (Table 2). The culture supernatants were harvested and hFGF23 protein was purified by ion exchange as previously described. The purified protein was tested in comparison with a commercial mammalian HGF23 on Laison CLIA analyzer using an automated prototype immunoassay (Diasorin) (Fig. 8).

Conclusions

PiggyBac transposable elements provide a powerful and efficient method for the establishment of stable CHO-cell lines for recombinant proteins production.

→ PB transposition increases up to 10-fold the generation of stable translocated clones compared to standard gene delivery strategy, also shortening the establishment process

→ Changing PB promoter strength enhances the frequency of high-producer clones

→ Exploiting this system we are able to generate hFGF23 producing CHO cell lines with an average yield of 20mg/L in batch culture

→ The obtained purified protein is correctly detected by both commercial ELISA assay and CLIA automated Diasorin immunoassay with comparable results to commercial mammalian HGF23.

References: