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D - - Expression of human prolactin in HEK293T using different transfection reagents

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INTRODUCTION

Prolactin is a hormone produced by the pituitary gland with numerous functions, such as lactation, reproduction, osmotic and immune regulation. This hormone is upregulated in cases of lack of lactation, infertility and cancer. Recombinant prolactin has been produced in *Escherichia coli* with an initial methionine which may cause immunological reactions, or in its authentic form in mammalian cells. Our laboratory has already synthesized human prolactin (hPRL) without initial methionine in *E. coli* periplasm and Chinese hamster ovary cells. CHO cells have been widely used in the synthesis of human recombinant proteins because of their similarity with human post-translational modifications as glycosylation. The HEK293, a human embryonic kidney cell, can do diverse glycosylation depend on culture conditions.

OBJECTIVES

This work compares different transfection reagents in the production of hPRL in HEK293T.

MATERIALS AND METHODS

The hPRL cDNA was inserted into the pEDdc vector donated by the Genetics Institute, USA. Three transfection reagents were used: LipofectamineTM (Thermo), XfectTM (Clontech), and ExpiFectamineTM (Thermo). HEK293T cells, a human strain, were cultured in 10 cm² Ø petri dishes with RPMI 1640 medium with 10% fetal bovine serum (FBS). After transfection, the medium was changed to serum free CHO-S-SFM II (Invitrogen, USA). 100% of the medium was collected and changed every two days. The collected medium was stored at -80°C. Samples were analyzed by SDS-PAGE, Western blotting and HPLC.

DISCUSSION AND RESULTS

The glycosylated and non-glycosylated hPRL forms secreted into the culture medium were confirmed by Western blot and RP-HPLC in the three transfected cultures in recombinant human cells. The reagent with the best result was Xfect (2 µg/mL), followed by Lipofectamine (1.6 µg/mL) and Expifectamine (1.2 µg/mL).

CONCLUSION

The transient expression of hPRL using HEK293T cells enable laboratory production of glycosylated hPRL for future studies of N-glycans produced by these cells.

Keywords: Prolactin, Transfection reagent, HEK293

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