Effect of promoter usage on recombinant eCG expression levels in stably-transfected CHO-K1 cells

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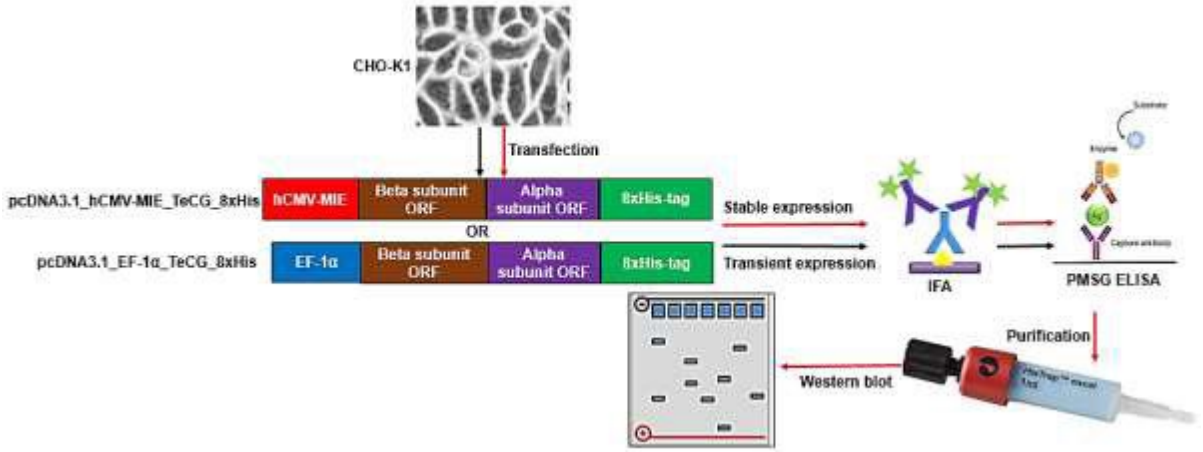

Recombinant expression of eCG in CHO-K1 cells using EF-1α and hCMV-MIE promoters

Introduction: Equine chorionic gonadotropin (eCG) or pregnant mare serum gonadotropin (PMSG) is a glycoprotein hormone that displays both follicle stimulating hormone (FSH) and luteinizing hormone (LH) activities in non-equids and is used in livestock animal reproduction. Its production solely depends on the extraction from the blood of pregnant mares, with disadvantages including potential blood contaminants, animal welfare issues and inter batch variations since glycosylation profile (which considerably influences the half-life and, consequently, the efficacy of the hormone) varies between sera at different stages of gestation. This study aims to produce a recombinant eCG (reCG) to substitute PMSG. Prolonged and high-level stable expression are required for therapeutic protein production in mammalian cells. Two commonly used promoters, human elongation factor 1 alpha (EF1α) and the human cytomegalovirus major immediate early (hCMV-MIE) were evaluated for enhancing recombinant tethered eCG expression levels in stably transfected CHO-K1 cells.

Methodology: The open reading frames (ORFs) encoding the alpha- and beta subunits of eCG were used to construct tethered eCG expression vectors, pcDNA3.1_hCMV-MIE_TeCG_8xHis and pcDNA3.1_EF-1α_TeCG_8xHis. Tethering of the expression vectors were achieved by fusing the 5' end of the alpha-subunit ORF to the 3' end of the beta-subunit. The tethered eCG constructs were transfected into CHO-K1 cells for transient and stable expression. An indirect immunofluorescence assay (IFA) targeted the 8xHis-tag on the C-terminal end of the tethered eCG protein was done to assess expression. Transient and stable expression were assessed after 48 hours via eCG ELISA. Purification was done using a HisTrap 1 ml column and western blot analysis was done to verify the size of the recombinant protein.

Results: Successful transient and stable expression was observed as confirmed by IFA and ELISA. Based on ELISA results, expression levels were higher under the control of EF-1α promoter than hCMV-MIE promoter during stable expression. The C-terminal, 8xHis-tagged, tethered eCG could be detected via western blot and the apparent molecular weight was about 55 kDa.

Discussion and Conclusion: The EF-1α promoter gave a higher expression level than the hCMV-MIE promoter suggesting that the EF-1α promoter would be valuable when developing vectors for generation of stable CHO-K1 cell lines expressing eCG stably and with high productivity. Lastly, the recombinant eCG represents a potential PMSG substitute which will eliminate issues around PMSG extraction from blood.

Keywords: equine chorionic gonadotropin, pregnant mare serum gonadotropin, recombinant eCG, promoter