Original Paper

Cloning and expression of human recombinant erythropoietin in *Leishmania tarentolae*

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Abstract

Background and Objective: Human erythropoietin (EPO) is a glycosylated hormone with molecular weight of about 40 KDa which is synthesized in kidneys and plays an important role in proliferation and differentiation of erythrocytes.This study was done to assess and analyze the expression of recombinant EPO in *Leishmania tarentolae* host.

Method: In this descriptive study, the EPO gene was codoned , optimized with bioinformatics database prior to be synthesized. It was cleaved by KpnIandXbaI enzymes and cloned into pLEXSY expression vector. The constructed expression cassette was transfected into *Leishmania tarentolae* through electroporaton method. Identification and confirmation of transfected colonies was performed using PCR expression diagnostic primers and EPO specific primers. Induction of the cloned gene was done with tetracycline. The expression in induced strains was analyzed by SDS-PAGE and western blotting techniques. The amount of recombinant protein was quantified by ELISA method. Confirmation of cloning and EPO expression cassette was carried out through genetic engineering procedures.

Results: Expression analysis of transfected parasitic strain with SDS-PAGE and western blotting confirmed gene integration into chromosomal of host as well as expression. The optimal conditions for expression were found to be 10μg of tetracycline and 72h induction time. Molecular weight of expressed protein estimated to be 40 KDa and expression level was determined to be 12.4 mg/l which was equal to 1% of total protein mass.

Conclusion: EPO expression cassette for cloning and expression in *Leishmania tarentolae* was designed and protein of interest was successfully induced and identified *Leishmania tarentolae* can be used as a suitable host for production of recombinant EPO and this technology has a potential for localization.

Keywords: Erythropoietin, *Leishmania tarentolae*, Gene expression, Cloning

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