Use of human prothrombin pre-pro leader peptide to improve the production of functional human FIX in cultured Drosophila S2 cells

Samira Bahrami[1]*, Jafar Vatandoost[2], Alireza Zomorodipour[3], Mahmoud Ghaffari[4], Fariba Ataei[5]

1, 4- Biochemistry group, Institute of Biochemistry and Biophysics, Tehran University, Tehran, Iran

2- Biology group, Hakim Sabzevari University, Sabzevar

3, 5- Molecular Genetic group, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran

bahramy@ibb.ut.ac.ir, Zomorodi@nigeb.ac.ir

Factor IX is a plasma glycoprotein necessary for the blood coagulation pathway, for which γ-carboxylation is considered as a critical post translation modification, that is required for its biological activity. Due to the fact that the affinity of the prothrombin propeptide for the γ-carboxylase is less than the factor IX propeptide, it was thought that the replacement of the of factor IX propeptide with that of the human prothrombin might increase the γ-carboxylation rate, because of its higher substrate turnover. Considering the potential of Drosophila γ-carboxylase to produce functional FIX, a chimeric cDNA consisting of hFIX next to a human prothrombin propeptide was constructed and inserted in a pMT/V5-HisA as a metal inducible expression vector. The recombinant plasmid in parallel with a similar plasmid containing native FIX were separately transfected into the Drosophila Schnider (S2) cell lines transiently, based on a calcium phosphate method . During 72 hours, after copper-ion induction, secreted FIX in cultured media were evaluated by performing ELISA. Also APPT assay was performed for analyzing the biological activity of the expressed rhFIX. Secretion of active FIX increased up to 303 ng/ ml 10^6 cell for the construct with prothrombin pre-pro leader on the second day and decreased on third day. Whereas in the construct consisting of native FIX the highest expression level with 253 ng/ ml 10^6 cell was achieved on the third day. The results indicate in the potential of the human prothrombin pre-pro leader peptide for the improvement of a γ-carboxylated protein such as FIX.

Keywords: propeptide, prothrombin, blood coagulation, γ-carboxylase, Drosophila S2 cell