PRODUCTION OF A BIOLOGICALLY ACTIVE MINIMAL B DOMAIN HUMAN FACTOR VIII IN MAMMALIAN CELL LINES

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Deficiency in the coagulation factor VIII (FVIII) causes a genetic disorder known as hemophilia A, which is commonly treated by repeated infusions of purified hemoderivatives. To avoid the potential of viral infection by plasma-derived products, recombinant factor VIII (rFVIII) has become an important alternative for hemophilia therapy. Moreover, Brazil is totally dependent on these imported products, spending about 80 million dollars a year. In the present work we report the cloning and expression of a truncated version of rFVIII in Chinese Hamster Ovary (CHO), Baby Hamster Kidney (BHK) and Human Hepatocellular Carcinoma (HEPG2) cells. Gene fragments for FVIII were obtained by RT-PCR from a human liver cDNA library. Bioengineered heavy and light chains were cloned and further fused to generate a shortened B linked single chain rFVIII (4.3 Kb) that preserves the thrombin like protease processing sites. rFVIII was transferred to mammalian cells expression vector and used for transient transfection. Coagulation activity was measured until 72h post-transfection. HEPG2 was the best cell line to express the rFVIII, reaching approximately 0.9 IU/mL/24h, followed by BHK and CHO cells. Detection of significant FVIII activity in transient transfection suggests an efficient production and processing of the recombinant factor.

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