Synthetic Biology as a therapeutic approach to Haemophilia A

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Introduction

Haemophilia A is a genetic X-linked disorder that leads to a coagulation factor VIII (FVIII) deficiency. Replacement therapy with human plasma derived protein or recombinant protein are the only choices available. However, there is a high cost for the treatment. Using a human cell line that grouws *ad infinitum, with a safe lentivirus system to deliver the FVIII vector*, an optimized production of the protein could be reached. A large amount of protein available could lead to an accessible treatment to a large number of patients.

Objectives

The purpose of these study is to develop a FVIII molecule by means of synthetic biology, and reach stable levels of functional recombinant factor VIII in a human cell line.

Methods

A semi-synthetic lentiviral vector was constructed carrying the wild-type factor VIII cDNA, pLVmpsvFVIIIΔB-Neo (Figure 1), and it authenticity was confirmed by DNA sequencing and PCR techniques.

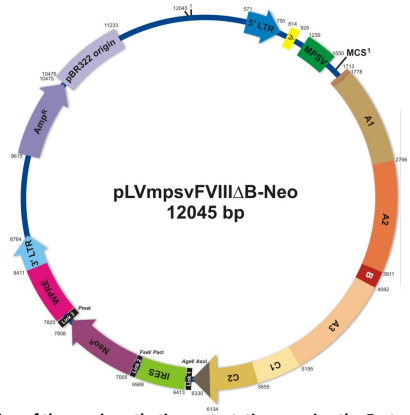

Figure 1 – Representation of the semi-synthetic construtction carrying the Factor VIII cDNA with a partial deletion of the B domain.

Results

To establish the functionality of the semi-synthetic vector LVmpsvFVIIIΔB-Neo the 293T cell line was transfected and, after 72 hours, were done the mRNA and functional FVIII protein analyses.

The 293T-syntFVIIIΔB cells showed levels of 17830.00 ± 3493.00X superior FVIII mRNA when compared to virgin cells (Figure 2a). Analyses of protein levels in supernatant of 293T-syntiFVIIIΔB cells presented 1,3 ± 0,01 IU/ml by chromogenic assay and 210ng/mL by the Enzyme-Linked Immunoabsorbent Assay (ELISA) (Figure 2b).

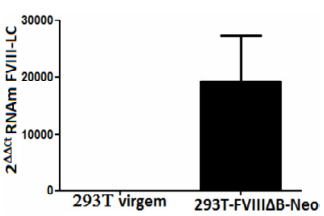

Figure 2a –Analyse of FVIII mRNA expression by Real Time qPCR.

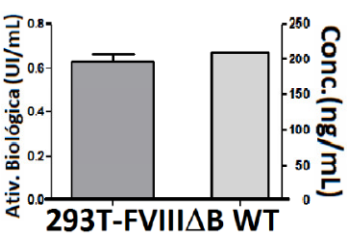

Figure 2b – Analyse of FVIII protein activity in the supernatant 72 hours after the transfection. By chromogenic assay (left) and ELISA (right).

The following step was the generation of a human cell line with a stable production of the wild-type FVIII. So, the human cell line Skhep was transduced with the semi-synthetic virus LVmpsvFVIIIΔB-Neo by a unique cycle and a multiplicity of infection (MOI) of 6.0, followed by a selection with geneticin during 18 days (Figure 3).

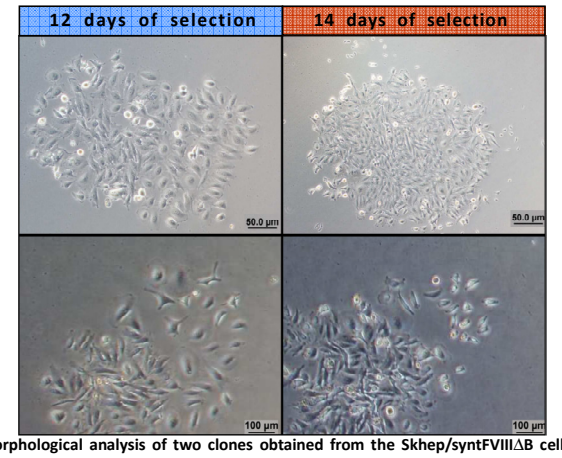

Figure 3 – Morphological analysis of two clones obtained from the Skhep/syntFVIIIΔB cell line. Above, the Skhep/syntFVIIIΔB clone 1.6. Below, the Skhep/sintFVIIIΔB clone 2.4 after 12 and 14 days of geneticin treatment.

The treatment with geneticin resulted in six cell clones and a mixed population of Skhep/syntFVIIIΔB cells with stable production of FVIII. So, they were characterized for: a) levels of relative FVIII mRNA (Figura 4); b) levels of FVIII protein biological activity (figura 5a) and 3) pro-viral copy numbers of FVIII cDNA/cell (Figura 5b).

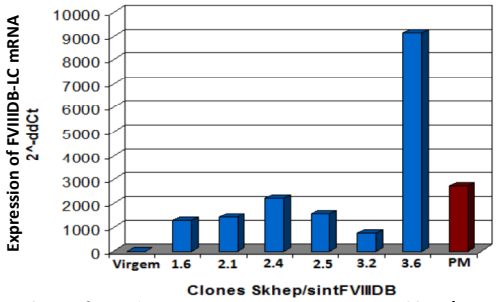

Figure 4 – Analysis of FVIIIΔB-LC WT mRNA expression in Skhep/syntFVIIIΔB clones and mixed population (PM). The cells showed levels of 757.87 to 9129.86X superior FVIII mRNA when compared to virgin cells.

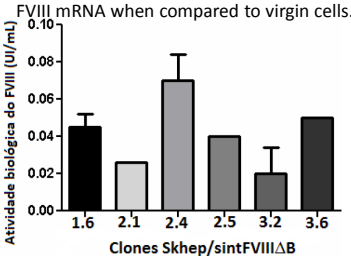

Figure 5a – Analysis of biological activity of synthetic FVIIIΔB-LC WT in the supernatant of the Skhep/syntFVIII-ΔB clones. By chromogenic assay the levels varied of 0.02 ± 0,01 IU/mL to 0.07 ± 0,01 IU/mL.

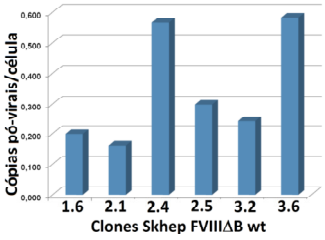

Figure 5b - Number of DNA copies integrated in the genome of the Skhep/syntFVIII-ΔB clones. All cell clones present low copy numbers: 0.19-0.58 pro-viral copies per cell.

Discussion

The date showed that the semi-synthetic construction is functional and allows the production of transitory levels of FVIII, which are similar to the human plasma, in the 293T cell line. It was also possible to obtain a cell population with stable production of FVIII, and current assays pretend to realize series of infection cycles with one of the Skhep/syntFVIIIΔB cellular clones. This will lead to the development of a human cell line with optimized production of FVIIIΔB to be used at the clinical practice.