

É necessário avaliar, de forma multidisciplinar, os resultados obtidos e traçar estratégias para o combate e eliminação desses patógenos, a fim de tornar a UTI um ambiente mais seguro e estável.

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A NOVEL SYNTHETIC AND RECOMBINANT GLUCOCEREBROSIDASE FOR GAUCHER DISEASE: IN SILICO MOLECULAR EVOLUTION AND GENE THERAPY APPROACHES TO ENHANCE ENZYME ACTIVITY

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Background: Gaucher disease (GD) is a rare lysosomal storage disease caused by pathogenic variants in the glucocerebrosidase gene (GBA1) resulting in a markedly decreased activity of the lysosomal enzyme b-glucocerebrosidase (GCase). Enzyme replacement therapy is the gold standard for treating GD patients. **Aim:** Here, we investigate whether *in silico* molecular evolution can be combined with synthetic biology and gene therapy for the development of a new recombinant enzyme with higher activity. **Materials and methods:** We developed novel GBA variants by introducing nonsynonymous mutations into the GBA gene obtained using *in silico* molecular evolution. These GBA variants are denoted as GBA-7, GBA-8, GBA-9 and GBA-12 and were cloned into lentiviral plasmids using synthetic biology approaches. Additionally, we obtained the wild-type GBA sequence from *Homo sapiens*, which is labeled GBA-Opt. In addition, we also aim to explore the differences in the transcriptional regulation using two

different promoters: the CMV and the human elongation factor 1A (hEF1A). As a control, we generated a construct that expresses the green fluorescent protein (GFP) gene under control of the same promoters. The characterization of these DNA constructs was performed using 293FT human cell line. We generated a total of 166 293FT cell lines with transient production of GBA (n = 100), GBA + GFP (n = 40), GFP (n = 8), and included virgin cells (n = 18). Out of the 100 GBA cell lines, 50 were utilized for real-time PCR analysis to investigate the transcription levels, while the remaining 50 were used to assess the enzyme-specific activity of GCase variants by fluorimetric assay. Furthermore, we determine mRNA secondary structures of GBA variants using the ViennaRNA and three-dimensional structures of the GCase-Opt and GCase-7 enzymes were elucidated using the AlphaFold2. **Results and discussion:** Quantitative real time PCR revealed that for 4 GBA transcripts (GBA-opt, GBA-7, GBA-9 and GBA-12) under control of hEF1A promoter showed higher expression in 293FT cells compared with CMV promoter (p < 0.05). Among the GBA variants, we observed that 293FT_GBA-7 cells express 5.2-fold higher transcript levels compared with 293FT_GBA-opt cells (p < 0.05). In cells lines co-transfected with GFP and GBA, we observed that comparing normalized activity values while considering transfection efficiencies can provide a more accurate assessment of the enzyme catalytic properties. We also screened the cell lysates for GCase specific activity. We observed that 293FT_GBA-7 cells and 293FT_GBA-Opt cells produced 507.6 ± 38.16 and 426.6 ± 25.25 nmol substrate hydrolyzed/mg protein/h, respectively. For the other transgenic 293FT cell lines these values were lower. Also, for 293FT transfected with mock plasmid this value was 82.92 ± 5.83 nmol/mg/h, respectively (p = 0.014). The 3D structures of GCase-Opt and GCase-7 revealed a high degree of alignment with the *Homo sapiens* enzyme (UniProt P04062). **Conclusions:** These findings demonstrate the promise of using *in silico* molecular evolution and synthetic biology approaches for developing enhanced enzyme variants for Gaucher disease, and suggest that GBA-7 could be a viable candidate for further investigation for replacement therapy in GD. Additional studies are warranted to investigate the biodistribution and long-term effects of GBA-7 in animal models.

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PERFIL DOS PACIENTES COM ARTROPATIA HEMOFÍLICA GRAVE SUBMETIDOS À CIRURGIA ORTOPÉDICA DA HEMORREDE DO CENTRO DE HEMATOLOGIA E HEMOTERAPIA DE SANTA CATARINA

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Objetivo: Descrever o perfil dos pacientes com artropatia hemofílica (AH) grave submetidos à cirurgia ortopédica na Hemorrede do Centro de Hematologia e Hemoterapia de