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

Santa Catarina (HEMOSC). **Materiais e métodos:** Trata-se de um estudo observacional transversal analítico realizado no HEMOSC, na cidade de Florianópolis/SC. Foram revisados os prontuários eletrônicos dos pacientes que foram submetidos a cirurgia ortopédica entre os períodos de agosto de 2019 a julho de 2023 e coletados os dados referentes a idade, tipo e gravidade da hemofilia, presença de inibidor, tipo de fator, tipo de profilaxia, comorbidade, local, estratégia e o tipo de cirurgia, amplitude de movimento (ADM), e índice de massa corporal (IMC). **Resultados:** As cirurgias ortopédicas ocorreram em 12 pacientes com diagnóstico de hemofilia A (91,7%) e hemofilia B (8,3%), 50% moderada e 50% grave, média de idade de 40,50 ± 9,97 anos, média de IMC de 26,33 ± 2,14, com sobrepeso (58,3%). O tipo de fator VIII mais frequente foi o fator plasmático (58,3%) e 8,3% fator IX. A presença de inibidor manifestou-se em três pacientes (25%), dos quais dois de baixo título (16,7%) e um de alto título (8,3%). As comorbidades incluíram hepatite C (25%), hepatite B (16,7%) e um (8,3%) soropositivo para HIV. A ADM do joelho e do quadril apresentou contratura de flexão (> 15%) em 90% dos casos no período pré-operatório. Foram realizadas 16 (80,0%) artroplastias totais de joelho (ATJ) e quatro (20,0%) artroplastias totais de quadril (ATQ), das quais apenas uma ATJ de revisão. Desses, seis foram ATJ bilateral (37,5%), quatro ATJ unilateral (25%), dois ATQ unilateral (50%) e uma ATQ bilateral (25%). Houve diferença estatisticamente significativa na ADM de extensão de joelho direito (p = 0,046) e esquerdo (p = 0,018) comparando-se antes e após a cirurgia. Não houve diferença estatisticamente significativa no arco do movimento (p = 0,255). **Discussão:** ATJ é a intervenção cirúrgica mais comum em pacientes com hemofilia como demonstrado no presente estudo. Já a ATQ é menos comum, mas o envolvimento do quadril compromete seriamente a qualidade de vida. A ADM pré-operatória é geralmente considerada o preditor mais importante do resultado funcional após ATJ, a maioria dos pacientes apresentou redução maior que 15% na flexão no pré-operatório, especialmente no joelho. Sabe-se que a rigidez pré-operatória do joelho, com uma ADM ≤ 50 pode prejudicar significativamente os resultados clínicos devido às características da hemofilia, como tendência ao sangramento, deformidade óssea prolongada, contratura dos tecidos moles e atrofias musculares que podem impactar no período pós-operatório. A ADM é uma preocupação no pós-operatório e geralmente é difícil de alcançar. Houve diferença estatisticamente significativa na ADM de extensão de joelhos direito e esquerdo comparando-se antes e após ATJ, demonstrando que a artroplastia pode auxiliar na redução da contratura muscular. **Conclusão:** O perfil dos pacientes identificados foi de hemofilia A com gravidade moderada ou alta, em adultos, com sobrepeso. O tipo de fator VIII mais frequente foi o plasmático. A maioria apresentou contratura de flexão maior que 15% no pré-operatório. Em relação à cirurgia prevaleceu a ATJ bilateral. Houve diferença estatisticamente significativa na ADM de extensão de joelho direito e esquerdo comparando-se antes e após a cirurgia. Este estudo, destaca-se por ser um trabalho pioneiro no estado do SC envolvendo cirurgias ortopédicas para este tipo de população.

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ANALYSIS OF THE *IN VIVO* FREQUENCY AND THE *IN VITRO* OSTEOGENIC DIFFERENTIATION OF IMMUNOPHENOTIPICALLY DEFINED HUMAN SKELETAL STEM AND PROGENITOR CELL SUBPOPULATIONS

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Skeletal stem and progenitor cells are responsible for bone maintenance and regeneration. Recently, a more specific set of phenotypic markers was described to identify the multipotential stem cell population (SSC, PDPN+ CD146− CD164+ CD73+), which gives rise to a PDPN+ CD146+ Bone, Cartilage and Stromal Progenitor (BCSP), which in turn originates a PDPN− CD146+ osteoprogenitor (OP) or a PDPN+ CD146− chondroprogenitor (CP). However, little is known about the frequency of these cell populations in bones of distinct anatomical locations, whether these phenotypic markers are stable enough to be used as distinctive markers during *in vitro* expansion, and whether these populations differ in osteogenic potential, three relevant questions for the development of characterized and effective Advanced Medicinal Therapeutic Cellular Products (AMTCPs) for application in innovative therapies for bone diseases. Therefore, the objective of this study was to investigate the frequency of SSCs and its progeny in the bone marrow collected from different bone sources and the stability of their immunophenotypic profile following *in vitro* expansion by FACS, their osteogenic differentiation potential *in vitro*, and how these profiles correlate with that of unfractionated Bone Marrow Stromal Cells (BMSCs). Following approval (n° 21768719.0.0000.5257), human hip (n = 19) and humerus (n = 5) samples were collected. In hip samples, SSCs accounted for 0.13% ± 0,20% of total nucleated non-hematopoietic cells, BCSPs were 4.78% ± 10.47%, CPs were 7.38% ± 14.12%, and OPs were 9.94% ± 12.20%. In humerus, SSCs and CPs were only detected in one sample (0,2% e 12%, respectively). BCSPs were 0.3% ± 0.15% and a higher frequency of OPs (25.05% ± 5.30%, p = 0.04) was detected in comparison to hip samples. Following PDPN− CD146+ (OP) and PDPN+ CD146− (SSCs and CPs) sorting, it was observed that their immunophenotypic profiles converged during expansion to PDPN+ CD146+, becoming similar to BMSCs. However, the expanded subpopulations differed in osteogenic capacity, with the PDPN+ CD146− (SSCs and CPs) producing a lower mineralized matrix area revealed by Alizarin Red staining than the BMSCs and OPs. Further *in vivo* functional assays will be performed to confirm these findings, that may lay the foundation to produce better AMTCPs.

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