

CLONING AND EXPRESSION ANALYSIS OF HUMAN
GLUCOCEREBROSIDASE (GCR) IN COS-7 CELLS USING ANTIBODIES
PRODUCED AGAINST THE RECOMBINANT GCR OF THE *E. COLI*

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Glucocerebrosidase (GCR) is a lysosomal hydrolase which degrades the glycolipid glucocerebroside to glucose and ceramide. Deficiency of this enzyme activity results in Gaucher disease, the most common lysosomal storage disorder. Currently available treatment for Gaucher disease include enzyme replacement therapy using a recombinant form of GCR expressed in CHO cells. In this work, the human GCR cDNA was cloned and the expression plasmids were functionally characterized by transient transfection before using the more laborious procedure of isolating and characterizing stable transfected cell lines. Thus, the GCR cDNA containing the own signal peptide or an Ig kappa-chain signal peptide were amplified by PCR, cloned in pED vetor which provides a high-level expression of heterologous proteins in mammalian cells and analyzed by transient expression in transfected COS-7 cells. The presence of expected protein band for the glycosylated GCR was detected by western blotting analysis using anti-GCR antibodies prepared in our laboratory against the recombinant protein expressed in *E. coli*. The subsequent steps include the stable transfection in CHO cells to obtain a productive cellular clone for production of GCR in large scale.

Supported by: FAPESP, CNPq and Fundação Butantan.