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TEMPORARY HIGH-LEVEL EXPRESSION OF BETA-GALACTOSIDASE ACTIVITY IN FIBROBLASTS FROM GM1 GANGLIOSIDOSIS PATIENTS

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GM1 Gangliosidosis is an autosomal recessive disorder caused by the deficiency of the lysosomal hydrolase acid b-galactosidase. The infantile form (GM1 type 1) is severe and shows neurodegeneration and visceromegaly that lead to death usually within two years. It's a lysosomal storage disorder frequent in Brazil, with a carrier frequency of 1:67. Both in vitro and animal studies showed the potential for gene therapy of this disease, as the enzyme is secreted and can be captured by deficient cells and targeted to the lysosomes. To the present, there is no effective treatment for GM1 Gangliosidosis. In order to test an expression vector for correcting the genetic defect of GM1 Gangliosidosis, we tested the gene transfer to fibroblasts in culture using liposomes. b-Gal cDNA was cloned into the expression vectors pSCTOP and pREP9. Transfection was performed using LIPOFECTAMINE 2000. After transfection, cells were harvested after 24h, 48h and 7 days. Enzyme activity was measured in cell lysate and supernadant by fluorometric assay. Treated cells 24 hours after transfection showed a much higher enzyme activity. (pREP9 b-Gal 1080.53, pSCTOP-b-Gal 1232.29, pREP9 B- gal + pTRACER 1259.63, pREP9 B- gal + pSCTOP B- gal 1718.94 nmoles/h/mgprot). However, cells maintained in culture for 7 days showed values similar to that of untreated patients. In this study we were able to transfect primary fibroblasts in culture using a non viral vector that over expresses the b-Galactosidase gene for a short period of time. To achieve a more prolonged effect, a different plasmid containing elements for nuclear retention and selective advantage should be used.