MOLECULAR BASIS OF HUMAN β-GLUCURONIDASE DEFICIENCY

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β-glucuronidase deficiency is a genetic disorder inherited as an autosomal recessive trait. It is due to the presence of an inactivated form of lysosomal acid hydrolase β-glucuronidase, which causes the accumulation of undegraded glycosaminoglycans in lysosomes and produces the mucopolysaccharidosis type VI disorder (MPS VI). Here we analysed several patients with MPS VI from the molecular level and have for the first time identified a mutation that gives rise to an inactivated form in a patient by cDNA cloning.

MATERIALS AND METHODS

For patients with MPS VI were studied to investigate the cause of this disease by Southern and Northern blot analysis. In addition, β-glucuronidase deficient fibroblasts from one patient were used in extracting total RNA with guanidium isothiocyanate, and poly (A)⁺ RNA was purified by application to Hybond-mAP affinity paper. The resultant mRNA was used to construct the cDNA library, which was screened by the plaque hybridization method. The probe for screening, which contained the full-length of the human β-glucuronidase, was kindly supplied by Dr. A. Oshima.

RESULTS AND DISCUSSION

DNAs from four patients and 15 unrelated healthy Japanese were digested with 12 different restriction enzymes, following Southern blot analysis. But the results show that there were no definite polymorphisms or deletions in all the patients and controls (data not shown).

Northern blot analysis of patients revealed normal transcripts which showed
that a promoter mutation was ruled out (Fig.1). Moreover, the molecular basis for enzyme deficiency has been determined by cDNA cloning. Twenty-three positive clones were obtained, by screening a total of $8 \times 10^5$ plaques from the original non-amplified library of which several overlapping clones covered the entire $\beta$-glucuronidase coding region (Fig.2). A single nucleotide substitution was confirmed by sequencing six cDNA clones derived from a patient. The mutation substitutes the amino acid valine for alanine at position 619 (CTG to GCG, Fig.3), and in COS cells transfected with the mutant cDNA the $\beta$-glucuronidase activity was not detected. Our study demonstrated the mutation responsible for $\beta$-glucuronidase deficiency.

REFERENCES