MUCOPOLYSACCHARIDOSIS TYPE VI: HETEROGENEITY OF DEFECTS IN
\( \beta \)-GLUCURONIDASE BIOSYNTHESIS

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Mucopolysaccharidosis type VI (MPS VI) is caused by genetic defects of lysosomal \( \beta \)-glucuronidase (\( \beta \) Gl). We previously identified several mutations causing \( \beta \) Gl deficiency. The cDNA clones of cases 1 and 2 contained a mutation (Ala\(^{576} \rightarrow \)Val) and that of case 3 contained mutation Arg\(^{882} \rightarrow \)Cys.\(^{12,20} \) In order to clarify the relation between cDNA mutation and protein biosynthesis, we here investigated the biosynthesis of \( \beta \) Gl proteins in pulse-chase experiments in both cultured skin fibroblasts and COS cells transfected with mutated \( \beta \) Gl clones from cases 1-3.

MATERIALS AND METHODS

Three Japanese patients affected with MPS VI were studied. The cells were maintained at 5% CO\(_2\) in MEM supplemented with 10% FCS. Transfection of the COS-7 cell was carried out as described elsewhere.\(^{20} \) Enzymatic activity was measured with 4-MU-\( \beta \)-glucuronide as a substrate. Labeled polypeptides were immunoprecipitated and characterized by SDS-PAGE followed by fluorography.

RESULTS AND DISCUSSION

The results indicate that normal \( \beta \) Gl was synthesized as a 82kDa premature form and then gradually converted into the 79kDa mature form in control fibroblasts, whereas no \( \beta \) Gl polypeptide was detected in any fibroblast from cases 1-3. In COS cells the mutant \( \beta \) Gl containing the Ala\(^{576} \rightarrow \)Val substitution was detected as a faint 82kDa premature band and gradually
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converted into 79kDa band, and the mutant β Gl containing the Arg\(^{282}\) → Cys substitution was not detected (Fig.1). As for mutant β Gl with the Ala\(^{619}\) → Val substitution, a little enzymatic activity was observed in transfected COS cells (Fig.2). We concluded from these results that each cDNA mutation has different effects on the biosynthesis and stability of the mutated β Gl in the transfected COS cells.

![Fig.1](image1)

Fig.1. Pulse-chase analysis of β Gl in cultured fibroblasts.

![Fig.2](image2)

Fig.2. Pulse-chase analysis of β Gl in transfected COS cells.

REFERENCES

1. Tomatsu S. et al. Gene 1990;89:283–287
