Molecular Basis for the Progeroid Variant of Ehlers-Danlos Syndrome

IDENTIFICATION AND CHARACTERIZATION OF TWO MUTATIONS IN GALACTOSYLTRANSFERASE I GENE*

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Progeroid type Ehlers-Danlos (E-D) syndrome was reported to be caused by defects in galactosyltransferase I (EC 2.4.1.133), which is involved in the synthesis of common linkage regions of proteoglycans. Recently, we isolated cDNA of the galactosyltransferase I (XGalT-1) (Okajima, T., Yoshida, K., Kondo, T., and Furukawa, K. (1999) J. Biol. Chem. 274, 22915–22918). Therefore, we analyzed mutations in this gene of a patient with progeroid type E-D syndrome by reverse transcription polymerase chain reaction and direct sequencing. Two changes of G and T to A and C at 186 and 206, respectively, were detected. Then, we determined the genomic DNA sequences encompassing the A186D and L206P mutations, revealing that the unaffected parents and two siblings were heterozygous for either one of the two different mutations and normal, while the patient had both of two different mutant genes. Enzymatic functions of cDNA clones of XGalT-1 containing the individual mutations were examined, elucidating that L206P clone completely lost the activity, while A186D retained 50% or 10% of the activity when analyzed with extracts from cDNA transfectant cells or recombinant soluble enzymes, respectively. Moreover, L206P enzyme showed diffuse staining in the cytoplasm of transfectant cells, while the wild type or A186D clones showed Golgi pattern. These results indicated that the mutations in XGalT-1 were at least one of main molecular basis for progeroid type E-D syndrome.

Glycosaminoglycans (GAGs)1 are carbohydrate structures of different length, different types, and varying numbers on proteoglycan molecules (1). GAG synthesis is initiated by the transfer of xylose onto serine residues in core proteins. Sequential addition of two galactoses, and a glucuronic acid forms a common linkage structure detected in major proteoglycans. Alternative addition of GlcNAc or GalNAc residues to the common linkage structure leads to the formation of heparin/heparan sulfate or that of chondroitin sulfate/dermatan sulfate, respectively. Defects in the pathway of GAG synthesis should, therefore, cause serious abnormalities in a wide variety of tissues and organs, since proteoglycans are ubiquitously present and are thought to be involved in the regulation of cell proliferation/differentiation, tissue development and organogenesis (2), and infections (3).

Progeroid type Ehlers-Danlos (E-D) syndrome (OMIM 130070) was reported to be caused by defects in a glycosyltransferase, i.e. galactosyltransferase I (4) (EC 2.4.1.133), which is involved in the synthesis of common linkage regions of proteoglycans. Recently, we have isolated a cDNA of the galactosyltransferase I gene (XGalT-1) (5) from a cDNA library of a human melanoma cell line based on the search of expressed sequence tag data base. This gene showed high homology (38%) to Caenorhabditis elegans sqc-3 gene (6), and its product showed specific activity of galactosyltransferase upon p-nitrophenyl-β-D-xlylopyranoside with β1,4 linkage. Moreover, expression of the cDNA in the mutant CHO cells deficient of heparan sulfate resulted in the restoration of the expression of both heparan sulfate and chondroitin sulfate, indicating that the gene codes XGalT-1. Thus, availability of this gene enabled us to elucidate the molecular basis of progeroid type E-D syndrome.

In the present study, we report two different missense mutations in the XGalT-1 gene of a patient with progeroid type E-D syndrome, both of which were derived from his parents. We confirmed the functional defects of those cDNAs harboring individual mutations by introducing into XGalT-1 deficient mutant CHO cells. Moreover, we analyzed the alteration in the intracellular localization of the mutant enzymes. These results have clearly elucidated molecular basis for the defects in the GAG synthesis, and indicated the mechanisms for the pathogenesis of progeroid type E-D syndrome.

EXPERIMENTAL PROCEDURES

Patient and His Family—The clinical symptoms and signs of the patient with progeroid E-D syndrome were by Kresse et al. (7). Briefly, the patient exhibited an aged appearance, developmental delay, dwarfism, craniofacial disproportion, generalized osteopenia, defective wound healing, hypermobile joints, hypotonic muscles, and loose but elastic skin. Enzyme assay of galactosyltransferase I revealed a marked reduction in galactosyltransferase I activity in the patient’s fibroblasts (less than 10% of normal), and a moderate decrease in the parents (~50% of normal) (4).

Cell Culture—Mouse fibroblast L cells and Chinese hamster ovary cells (CHO-K1) were grown in Dulbecco’s modified Eagle’s minimum essential medium supplemented with 7.5% fetal calf serum at 37 °C in a 5% CO2 atmosphere. CHO mutant pgsB-781 (8) was obtained from the American Type Culture Collection and grown in F-12 medium (Life Technologies, Inc.) supplemented with 10% fetal calf serum.

Sequence Analysis of XGalT-1 Gene—Established skin fibroblasts from the patient and his family were used as a source of RNA and DNA

CHO, Chinese hamster ovary; mAb, monoclonal antibody; PCR, polymerase chain reaction; PBS, phosphate-buffered saline; FITC, fluorescein isothiocyanate; WT, wild type; MES, 4-morpholineethanesulfonic acid.

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1 The abbreviations used are: GAGs, glycosaminoglycans; E-D syndrome, Ehlers-Danlos syndrome; XGalT-1, galactosyltransferase I;
for mutation analysis. The entire coding region of XGalT-1 was amplified by polymerase chain reaction (PCR) of cDNA prepared from total RNA as described previously (5). PCR was performed with primers XGT-20 (5′-ATGCGCCCGCCGCCTCCGGCA-3′) and XGT-3GSP1 (5′-GCCACTCCACATCTGTCAG-3′). The products were subcloned and sequenced by the dideoxy termination method using an ABI PRISM® 310 Genetic Analyzer (Applied Biosystems). To perform pedigree analysis, DNA segments encompassing the A186D and L206P mutations were amplified from genomic DNAs by PCR using the primers XGT-F460 (5′-AACAGCACCGACTACATTGCC-3′) and XGTR622 (5′-CAGCGTATGCTGTCTT-3′). The amplified products were directly sequenced using primer XGT-F460 as described above.

Construction of Expression Vectors—Truncated form of XGalT-1 lacking 53 amino acids from the N terminus, was prepared by PCR using a 5′ primer containing an EcoRI site, 5′-CACGCTGAAATCTCTTGCGGA-GGTGGCCCGGG-3′, and a 3′ primer containing a XhoI site, 5′-TGGCC-ACTCGAGTCGACTGATGTGGCACCAC-3′ (nucleotides 1007–1024), and subcloned into EcoRI and XhoI sites of pcD-SA vector (kindly provided by Dr. Tsuji) as described (5). Full-length coding region of the EcoRI and subcloned into pBSK+ (Stratagene, La Jolla, CA) using a PCR product of 8.3 kb that encompasses the entire coding region of XGalT-1 was amplified by reverse transcription PCR and sequenced.

The antibody binding was revealed with ABC-PO (Vector) and HRP-conjugated goat anti-mouse IgG, then observed under MRC-1024 confocal imaging system (Bio-Rad).

Western Blot Analysis—PgsB-761 cells transfected with 10 μg each of Myc-tagged XGalT-1 and mutants were lysed in 100 mM MES buffer (pH 6.0) containing 1% Triton X-100, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, and total cell lysate (50 μg) were separated by SDS-polyacrylamide gel electrophoresis and immuno-blotted with mouse anti-Myc tag antibody and biotinylated horse anti-mouse IgG. The antibody binding was revealed with ABC-PO (Vector) and HRP-1000 (Konica, Tokyo) according to the manufacturer’s instruction.

Preparation of Soluble Forms of XGalT-1—Cell lysates of the transfected cells were centrifuged at 15,000 × g for 10 min, and the supernatant was collected as the supernatant fraction. The pellet was resuspended in 1% SDS and boiled for 1 min, and the supernatant was collected as the insoluble fraction.

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Fig. 1. Identification of XGalT-1 mutations. Pedigree and sequence analysis of genomic DNAs from the patient’s family members. DNA segments were amplified from genomic DNAs by PCR as described under “Experimental Procedures.” The amplified products were directly sequenced using primer XGT-F640. The C → A transition changes the sequence of codon 186 from GCC to GAC and resulted in an Ala to Asp substitution. The T → C transition changes codon 206 from CCC to CCC, resulting in a Leu to Pro substitution.

RESULTS AND DISCUSSION

Mutations in XGalT-1 Gene in the Patient with Progeroid Type of E-D Syndrome—The entire coding region of XGalT-1 was amplified by reverse transcription PCR and sequenced. Just two changes of G and T to A and C at 186 and 206, respectively, were detected (Fig. 1). To perform pedigree analysis, we determined the genomic DNA sequences encompassing the A186D and L206P mutations. Consequently, comparison of the genomic and cDNA sequences indicated that two mutations are present in the same exon (Fig. 2). As shown in Fig. 1, the unaffected parents and two siblings were heterozygous for either one of the two different mutations and normal, while the patient had both of two different mutant genes.

Functions of the Products from Mutant cDNAs—To confirm whether the A186D and L206P mutations were responsible for the defects in the synthesis of GAGs, we examined the functions of the expressed mutant cDNAs. XGalT-1-deficient mutant CHO line pgsB-761 (8) was transfected with WT, A186D and L206P cDNAs to examine the restoration of the expression of proteoglycans. A186D could restore fairly well and similarly to WT, while L206P could not at all (Fig. 3A). The enzyme activities in the total cell lysate of these transfectant cells were measured in a liquid scintillation counter (Beckman).

Furthermore, soluble enzymes fused to protein A were ex-
pressed in L cells and were concentrated 100-fold and served for enzyme assay. Again, A186D showed low activity and L206P showed no activity (Fig. 3B, right).

Intracellular Localization of the Mutant Enzymes—Intracellular localization of the mutant enzymes derived from the defined mutant cDNAs was analyzed using Myc-tagged constructs. WT and A186D enzymes showed a similar Golgi pattern. In contrast, L206P was detected in the cytoplasm with a diffuse pattern (Fig. 4). Although the disruption of Golgi targeting of L206P enzyme is an interesting finding, it seems not direct mechanisms for the functional defects as a galactosyltransferase, because the soluble L206P enzyme also completely lost the enzyme activity.

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