シンポジウム I. 優性遺伝性疾患の最近の知見

Symposium I. Autosomal Dominant Disorders: Recent Advances

Chairpersons: Masataka ARIMA (Tokyo) and Hideo HAMAGUCHI (Tsukuba)

SI-1. MOLECULAR ANALYSES OF FAMILIAL AMYLOIDOTIC POLYNEUROPATHY.

Shuichiro MAEDA and Kazunori SHIMADA (Dept. Biochem., Kumamoto Univ. Med. Sch., Kumamoto)

Familial amyloidotic polyneuropathy (FAP) is an autosomal dominantly inherited systemic amyloidosis, characterized by prominent peripheral nerve involvement, and by the extracellular deposition of fibrillar amyloid protein. The amyloid deposit consists mainly of a variant transthyretin (prealbumin) with a single amino acid substitution and contains a small amount of serum amyloid P (SAP) component. The symptoms are first recognized when the patient is between 20 and 45 years of age, and the disease is progressive and death follows after about 10–20 years. To investigate the molecular pathogenesis of FAP, we carried out experiments as follows.

1) The DNA diagnosis of FAP was established. This approach clearly revealed a direct link between the transthyretin gene mutation and FAP.

2) The human normal transthyretin genomic DNA and a mutant transthyretin genomic DNA assiocated with FAP were isolated. The nucleotide sequence of the mutant gene was in complete agreement with that of the normal one, except for a single-base substitution present in the second exon. This substitution is responsible for the transition from valine to methionine at position 30, in the mutant transthyretin gene.

3) Levels and distribution of transthyretin mRNAs in various tissues of control subjects and those of individuals with FAP were analyzed. Transthyretin mRNAs were detected in the total RNAs from liver and choroid plexus of brain, but not in those from the other part of brain, heart, thyroid gland, and kidney. The levels of transthyretin mRNAs in the livers of control subjects and those of individuals with FAP were much the same. Because all the individuals with FAP, so far examined, were heterozygous for the transthyretin gene, the levels of the normal and mutant transthyretin mRNAs in the liver and the brain tissue containing choroid plexus were separately estimated and were found to be approximately equal.

4) Mouse transthyretin cDNA and genomic DNA were isolated and their nucleotide sequences were determined. Certain regions of their structures were revealed to be highly conserved in mice and humans.

5) The mouse transthyretin gene expression during development was investigated using *in situ* hybridization procedures. RNAs hybridizing to the mouse transthyretin cDNA were detected as early as at the 10th day of gestation, and at that time were specifically localized in endodermal cells of the visceral yolk sac, tela choroidea (the predecessor of the choroid plexus), and hepatocytes. In the adult mice, transthyretin mRNA was localized in the hepatocytes, choroid plexus epithelial cells, and retinal cells.

6) Human and mouse SAP cDNAs and genomic DNAs were isolated. The deduced amino acid sequence of human SAP is highly homologous with that of mouse SAP.

7) The level of SAP mRNAs in mouse liver increased up to 60-fold during the first 20 hr after induction of an acute inflammation.

8) Transgenic mice carrying and expressing the human mutant transthyretin gene were constructed. In these mice, human transthyretin and mouse SAP were deposited as amyloid fibrils.

SI-2. MUTATIONS IN COLLAGEN METABOLISM DISORDERS. Hiroshi KONOMI (NCNP-Natl. Inst. Neurosci., Tokyo)

Collagen is one of the major components of extracellular matrix. There are 13 different types of collagen molecules, and at least 24 genetically distinct subunit chains.

Characteristic clinical features of collagen metabolism disorders are deformities of skeleton, bone fracture, fragility and hyperextensibility of skin, joint hypermobility, easy bruisability, venous varicosities, hernia and ocular fragility. Collagen metabolism disorders include Ehlers-Danlos syndrome (ED), osteogenesis imperfecta (OI) and Marfan syndrome (MF). ED and OI show large heterogeneity in clinical manifestations. Recent advances of collagen metabolism revealed that pathogenesis of ED are considered to be abnormalities of biosynthetic processes of type I and III procollagen or that of maturation of type I collagen fibers with a few exceptional variants, and that of OI are type I procollagen gene mutations. On the other hand, in spite of researches about one MF variant who showed insertion of about 20 amino acids in α2(I)-CB5 of type I collagen, underlying defects in connective tissues of MF is still unclear.

One of the characteristic features of osteogenesis imperfecta with collagen gene mutations depend on triple herical structure of type I collagen molecule, which is composed with two α1(I) chain and one α2(I) chain. When cultured fibroblasts from the patients synthesized approximately equal amounts of normal proα1(I) chains and of shortened proα1(I) chains (mutant proteins), one-half of the trimers assembled in cells contained one shortened proα1(I) chains and one-quarter contained two shortened proα1(I) chains. The trimers containing the shortened proα1(I) chains, however, were metabolically unstable. They folded into a triple-helical conformation only at temperatures well below body temperature. The phenomenon, which has been referred to as "protein suicide," created a situation in which the total amount of biologically useful type I procollagen produced by fibroblasts was reduced to one-quarter of the control.

We analyzed collagens produced by skin fibroblasts from a patient with a lethal form variant of osteogenesis imperfecta (OI-type II). They synthesized equal amount of two proα1(I) chain, one was normal and the other was shortened chain. CNBr peptide mapping, animal collagenase digestion revealed that the mutation location of the shortened proα1(I) chain existed in CB8-peptide. In addition to the mutation of proα1(I) chain, there was elevated synthesis of type IV and V collagen. These results indicated that alteration of collagen metabolism occurred in the disease.