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REVIEW

EPIDERMOLYSIS BULLOSA : DIAGNOSIS AND PRENATAL DIAGNOSIS

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Recent advances in the diagnosis and prenatal diagnosis of severe forms of epidermolysis bullosa (EB) have been reviewed. Using electron microscopy and immunohistochemistry of specific monoclonal antibody, foetal skin biopsy during the second trimester of pregnancy has been utilized successfully for the prenatal diagnosis of EB. Recently, elucidation of the specific gene mutation in affected individuals allowed us to perform DNA-based prenatal diagnosis during the first trimester of pregnancy. Our own experience with prenatal diagnosis of EB at the Special Clinic for Inherited Skin Disorders at Keio University Hospital for the last six years is summarized.

Key Words : Epidermolysis bullosa, Foetal skin biopsy, DNA-analysis, PCR, Basement membrane

Introduction

Foetal skin biopsy has played an important role in the development of prenatal diagnosis of certain genetically determined skin disorders during the past decade, include epidermolysis bullosa, oculocutaneous albinism, Harlequin ichthyosis, lamellar ichthysosis, bullous congenital ichthyosiform erythroderma, anhidrotic ectodermal dysplasia and incontinentia pigmenti.[1-4] Foetal skin sample can be examined by light and electron microscopy for morphological, biological and immunohistochemical abnormalities. Nevertheless, skin biopsy has the disadvanatges of being performed only in the second trimester of pregnancy and of requiring a long waiting period for test results. By this time too, an at-risk woman may have already experienced prolonged anxiety about the status of her baby. Thus, a number of investigators have sought a method by which to perform prenatal diagnosis in the first trimester. Advances in molecular biology have elucidated the specific mutant gene responsible for some of the genodermatoses, allowing for DNA-based prenatal diagnosis of certain inherited skin disorders that can introudce chorionic villi sampling or amniocentesis in the earlier stage of pregnancy.

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Diagnosis and prenatal diagnosis of epidermolysis bullosa (EB)

Epidermolysis bullosa is an inherited skin disease that encompasses more than twenty subtypes having the common characterisitic of marked skin fragility and blister formation after seemingly minor or insignificant trauma to the skin.[5] There are three major types of epidermolysis bullosa : simplex, junctional and dystrophic type. These are classified based on where the blisters form ultrastructurally withing the skin following mechanical trauma. In the epidermolytic form referred to as EB simplex, skin cleavage or blister formation occurs within the lower portion of the epidermis. In junctional EB, blisters arise within the lamina lucida. As a result, the intact epidermis forms the roof of the blister while the lamina densa remains along its base. In the dermolytic from of inherited EB, referred to as dystrophic EB, skin cleavage occurs

beneath the lamina densa; therefore, the epidermis and intact basement membrance form the roof of the blister while the denuded dermis forms its base.

Technologic advances of the study of normal human skin has led to a greater understanding of the pathogenesis of various forms of inherited EB.[3] In particular, the generation of monoclonal antibodies specific for skin basement membrance has led to the discovery of several selective antigenic defects in the skin from patients with various forms of EB.[6] These antibodies provide additional confirmation that certain subtypes have similar or identical phenotypes during the neonatal period.[7-9] Clinical features of EB variants greatly differ from one another. Prenatal diagnosis of three of most severe forms of EB variants with autosomal recessive inheritance, including lethal junctional EB of Herlitz type, recessive dystrophic EB and junctional EB-pyloric atresia syndrome, has been the standard practice in dermatology for the last 15 years.

1. Lethal junctional EB of Herlitz type (Herlitz EB)

Clinical features of Herlitz EB, autosomal recessive inherited genodermatoses, are widespread blisters, erosions and the development of large, non-healing areas of granulation tissue. The prognosis is very poor and the majority of patients die before one-year of age, although there have been a few exceptions. The prenatal diagnosis of this condition with autosomal recessive inheritance was first performed in 1980 using skin samples from an 18-weeks estimated gestational age foetus.[10] Herlitz EB is the most commonly diagnosed genetic skin disease in utero using the foetal skin biopsy and is the first variant among EB whose prenatal diagnosis was performed in Asia.[11]

Although structural abnormalities of the hemidesmosomes and specific antigen expression occur in the earlier weeks of gestation, the procedure is postponed until the second trimester due to difficulty in sampling and handling of the tissue at the earlier weeks. Until the mid 1980s, the primary criteria for diagnosis of the affected foetal skin was the separation of the epidermis in the plane of the lamina lucida and the hypoplastic development of the hemidesmosomes.[2,12] The GB3 monoclonal antibody,[13] directed against nicein/kalinin/epiligrin or laminin 5 in the new nomenclature,[14] has been shown to be specifically absent in the skin of Herlitz EB and can be used as a prenatal diagnostic probe.[7]

In 1990, the Special Clinic for Genetic Counselling on Inherited Skin Diseases at Keiol University was opend and in 1991, we performed the first successful trial of prenatal detection of EB in Asia.[11] In this Japanese family with Herlitz EB, the proband, who died a few months after birth, showed the formation of generalized bullae. Electron microscopy of the skin of the proband showed dermo-epidermal separation at the lamina lucida and complete negative staining with the GB3 monoclonal antibody against laminin 5. The parents opted opted for prenatal diagnosis with the next pregnancy, and the foetal skin biopsy was performed at 19 weeks of gestation under the ultrasound guidance.[15] Of the four small skin samples (<1 mm^3), two were processed for electron microscopy and the rest were used for immunohistochemistry. Electron microscopy showed no dermo-epidermal separation with mature hemidesmosomes. Indirect immunofluorescence revealed a normal bright basement membrane staining of GB3 monoclonal antibody, indicating that the

foetus was not affected.[11]

GB3 antigen or laminin 5 in the new nomenclature, known to be absent in Herlitz EB, consists of three polypeptide subunit chains, α3 (150 kDa), β3 (125 kDa) and γ2 (100 kDa), encoded by the distinct gene LAMA3, LAMB3 and LAMC2, respectively.[14] Specific mutations in both the LAMB3[16] and LAMC2[17] genes have been reported in patients with Herlitz EB. Cloning of the full-length cDNAs encoding the three chains of laminin 5 and identification of the mutations in the corresponding genes provide a new means for direct DNA-based prenatal diagnosis using chorionic villus at 10-weeks gestation. DNA-based prenatal diagnosis was reported recently,[18] and expected to be further applicable for certain Herlitz EB families in the future. In September 1995, we indeed performed DNA based prenatal diagnosis for a foetus at rist of Herlitz EB, and diagnosed that the foeuts was not affected (Shimizu H et al; paper in preparation.)

2. Recessive dystrophic EB (RDEB)

Recessive dystophic EB, inherited as an autosomal recessive trait, includes the generalized mutilating form (Hallopeau-Siemens type) which is the most severe RDEB, the localized non-mutilating type (mitis), and the localized and inversa forms. The Hallopeau-Siemens type of RDEB (HS-RDEB) is responsible for extensive blistering of the skin and mucous membranes leading to mitten-like deformities of the hands and feet, loss of nails, joint contractures, and oesophageal stricutres. Malnutrition, anemia, growth retardation, and squamous cell carcinoma are frequent complications of HS-RDEB.[19] The morphologic criteria to recognize HS-RDEB in utero are based on observations of the skin from affected foetuses and postnatal individuals.[20] The first prenatal diagnosis of HS-RDEB was made by foetal skin biopsy.[21] Electron microscopy reveals separation of the epidermis from the dermis in the plane below the lamina densa and absent or a markedly reduced number of mature anchoring fibrils. Identificaiton of type VII collagen, as the major component of anchoring fibrils, was a critical step in investigating the pathogenesis of RDEB.[22] It allowed the development of a monoclonal antibody against the protein, such as LH7.2.[23] The epitope of LH7.2 was found to be localized within the NC1 domain of type VII collagen at both ends of the anchoring fibrils.[24-26] The majority of the HS-RDEB patients display the absence of staining of the epidermal basement membrane with LH7.2.[23] Thus, LH7.2 has been applied as a diagnostic probe for HS-RDEB.[6]

Recently, we accomplished the first prenatal diagnosis of HS-RDEB for a Japanese family by foetal skin biopsy using the LH7.2 as a immunohistochemical probe for detection.[27] In this family, electron microscopy of the skin from the proband demonstrated dermo-epidermal separation below the lamina densa and no mature anchoring fibrils. LH7.2 staining was completely negative at the epidermal basement membrane zone. The parents sought prenatal diagnosis for their next pregnancy. The foetal skin biopsy was performed and electron microscopy of the foetus revealed no dermo-epidermal separation and numerous mature anchoring fibrils. In addition, the foetal skin showed positive LH7.2 staining by indirect immunofluorescence. This suggested that the fotus was unaffected resulting in the birth of a healthy newborn.[27]

Recent cloning of COL7A1, encoding type VII collagen[28,29] and the identification of

gene mutations provide a new means for direct DNA-based prenatal diagnosis.[30-32] We succeeded in achieving DNA-based prenatal diagnosis for a Japanese family with RDEB both by amniocentesis and by chorionic villus sampling.[33] The proband was a 6-month-old Japanese male with typical clinical, histological, and ultrastructural features of RDEB with severe fusion of toes. Molecular analysis of COL7A1 gene revealed the proband and his father to be heterozygous for a 1 bp deletion of a C in exon 70 (5818delC), while the maternal mutation has not been identified. In addition, four microsatellite markers (D3S1029, D3S1235, D3S1076 and D3S1573) located in the DNA flanking the COL7A1 locus were shown to be informative for genotyping. In December 1994, the mother was in her second pregnancy and hoped prenatal diagnosis. Analysis of the foetal DNA extracted from amniotic cells obtained at 15 weeks of gestation indicated that the foetus was affected. The parents chose the termination of the pregnancy and the abortus was confirmed to be affected with RDEB. In July 1995, the parents had their third pregnancy and wished the DNA based prenatal diagnosis again. The analysis of the foetal DNA confirmed that the foetus has two normal alleles of COL7A1 and the pregnancy has been continued.[33] Genotype analysis with the COL7A1 mutation in families at risk for RDEB represents an early and rapid diagnostic alternative to second-trimester evaluation of foetal skin samples offiering a major advancement in prenatal diagnosis.[34]

3. Pyloric atresia-junctional EB syndrome (PA-JEB)

Pyloric atresia (PA) associated with epidermolysis bullosa (EB) is a distinct entity inherited as an autosomal recessive trait.[35-38] A recent review of this condition disclosed more than 40 such cases, including 6 pairs of siblings.[37] Most of these infants died in the first few months of life despite a surgical correction of the pyloric abnormality. PA is probably the primary event, rather than the result of scarring secondary to junctional EB. Therefore, all associated cases should be categorized as junctinal EB termed "PA-junctional EB syndrome (PA-JEB)."[37]

Previously prenatal diagnosis of PA-JEB was based on the ultrastructural findings within foetal skin of the intra-lamina lucida cleavage and hypoplasia of the hemidesmosomes.[35] Electron microscopy seems to be the only reliable method for prenatal diagnosis, because laminin 5, detected by GB3 monoclonal antibody, is expressed normally in PA-JEB and cannot be used as a diagnostic probe.[35]

To overcome this technical problem, we have applied the 19-DEJ-1 monoclonal antibody as an immunhistochemical probe for prenatal diagnosis.[38] In this Japanese family, both the first and second child was affected with PA-JEB and died within 2 weeks after being delivered. In her third pregnancy, the prenatal diagnosis was performed by foetal skin biopsy and electron microscopy demonstrated no apparent sign of dermoepidermal separation nor hypoplasia of hemidesmosomes. The 19-DEJ-1 monoclonal antibody showed bright linear staining at the epidermal basement membrane that was completely absent in the skin of the affected siblings. The foetus at risk was diagnosed as unaffected. The 19-DEJ-1 monoclonal antibody should be applied as a diagnostic probe for the prenatal diagnosis of PA-JEB.[38]

More recently, absence of b4 integrin, a component of hemidesmosome, was found in the skin of a patient of PA-JEB.[39] Subsequent study proved the presence of mutation of b4

integrin gene in PA-JEB.[40] On the contrary, we recently reported a Japanese patient with PA-JEB showing absence of detectable a6 integrin (manuscript in submission). These latest findings would indicate the genetic and phenotypic heterogeneity of PA-JEB.

Discussion

There are a large number of severe inheritable skin disorders that have been considered indications for prenatal diagnosis. Until 1980s when the first prenatal diagnosis for inherited skin diseases was introudced, however, most of the severe genetic disorders of the skin have not been able to be diagnosed prenatally. Many parents, who once experience the severity and course of one of these severe genodermatoses, are inclined to interrupt a new pregnancy to prevent its repeat, even though they wish to have another child. Thus, many healthy foetuses have been aborted because of the unavailability of the prenatal diagnosis.

Several genetic skin diseases are life threatening or have consequences that are so significant that the quality of life of affected individuals is severely compromised. For many families in which these disorders are expressed, there is a desire to recognize the condition of a foetus at risk. Some of these disorders can be detected through the use of cultured amniotic fluid cells, chromosomal analysis, assays of amniotic fluid, or blood. For other diseases, samples of foetal skin obtained between the 18 to 19 weeks of pregnancy are obtained by in utero biopsy and evaluated using morphologic, immunohistochemical, and biochemical techniques.[1] Linkage analysis and identificaiton of mutations for some of these diseases have permitted prenatal diagnosis to be accomplished in specific families using foetal DNA obtained from chorionic villi or amniotic fluid cells. Molecular analysis of foetal DNA is certainly the method of choice for prenatal diagnosis because it can be performed early in gestation and the results can be obtained in a day or two. However, this method is available for only a few of the diseases of concern and where mutations have been identified that they are family specific.[1] In sampling the foetal cells for DNA analysis, the possibility of the contamination of the maternal cells should always be considered. To exclude this possibility, culturing the part of foetal cells for the later back up analysis is always desired. On the contrary, the experience in prenatal diagnosis using foetal skin samples is internationally extensive, thus evaluation of foetal skin samples still remains a valid and an important procedure for distinguishing normal from affected foetuses in utero.

Until recent successful introduction of DNA based analysis,[30,32,33,41] all the previous prenatal diagnosis had been only made by foetal skin biopsy. Further advances in molecular study of each inherited skin disorders should replace foetal skin sampling by DNA based prenatal diagnosis. Furthermore, elucidation of detailed genotype/phenotype correlation in respective condition may help future introduction of gene therapy for the patient as well as for the affected foetus during gestation.

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