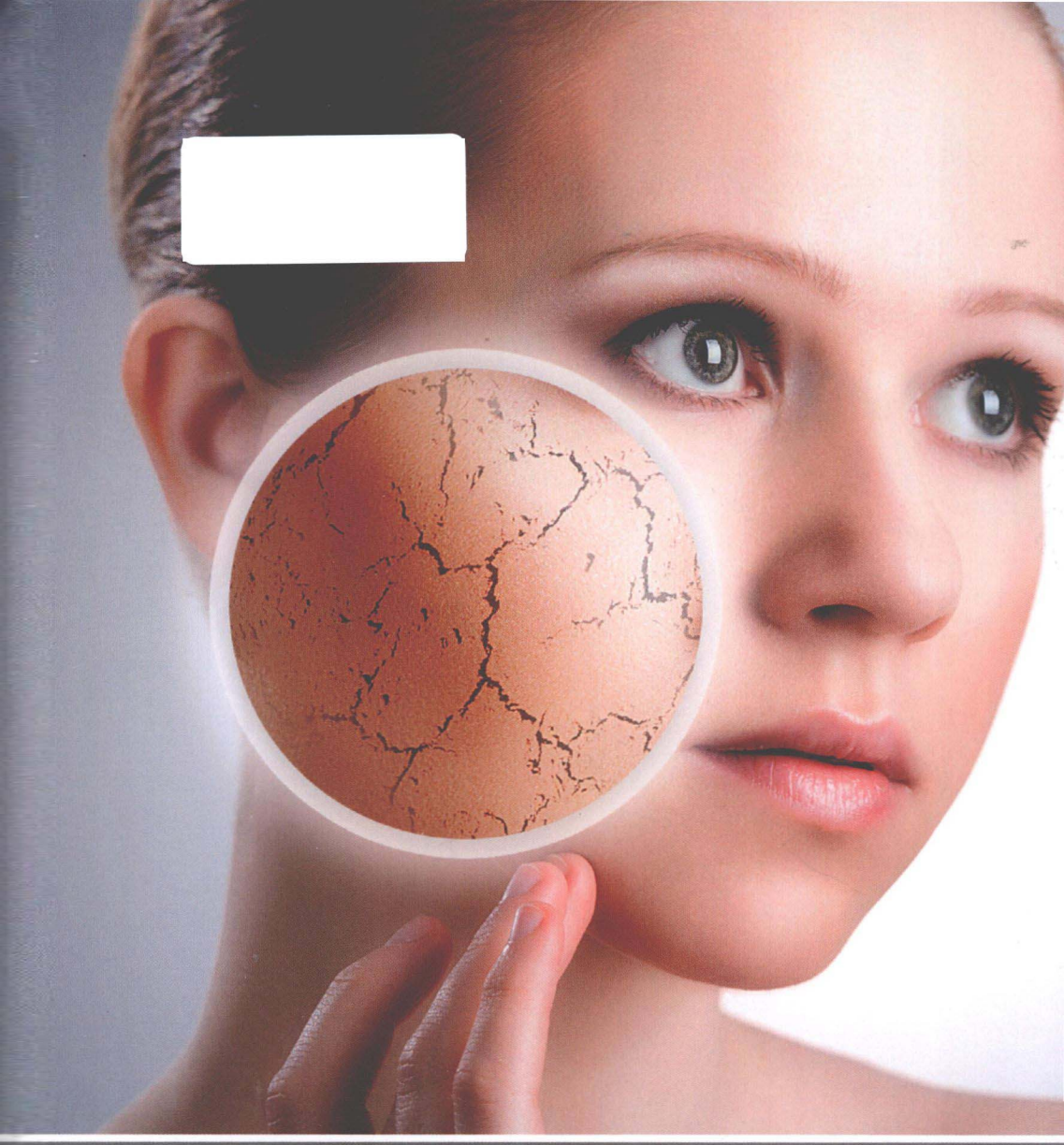

Dermatology

Genetics and Novel Findings

Deb Willis

Dermatology: Genetics and Novel Findings

Edited by **Deb Willis**

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Preface

The book provides comprehensive information highlighting recent developments in the field of dermatology. Several distinct skin diseases are inherited as Mendelian inheritance. The cause of genetic skin disorders is mutations in the genes encoding proteins expressing in skin, melanocytes, skin appendages and immune-related cells. Recognition of genes and explanation of function of the encoded proteins may provide latest techniques to tackle the disorders. The aim of this book is to provide latest information regarding every disorder to physicians, dermatologists and scientists, and offer new therapies to affected individuals.

The information contained in this book is the result of intensive hard work done by researchers in this field. All due efforts have been made to make this book serve as a complete guiding source for students and researchers. The topics in this book have been comprehensively explained to help readers understand the growing trends in the field.

I would like to thank the entire group of writers who made sincere efforts in this book and my family who supported me in my efforts of working on this book. I take this opportunity to thank all those who have been a guiding force throughout my life.

Editor

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Epidermolysis Bullosa Simplex

Ken Natsuga

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1. Introduction

Epidermolysis bullosa (EB) is a heterogeneous group of congenital disorders characterized by skin blister formation. EB is subdivided into three main subtypes (EB simplex (EBS), junctional EB (JEB) and dystrophic EB (DEB)) and one minor subtype (Kindler syndrome (KS)), according to the level of skin split [1].

The EBS subtype can be defined as EBS with blisters within epidermal basal keratinocytes or above, and it is distinguished from other subtypes whose levels of blister formation are deeper (JEB and DEB) or variable (KS). Mutations in several genes have been identified as being responsible for EBS phenotypes. The clinical manifestations of EBS vary greatly depending on the causative genes. Some EBS subtypes are mild and tend to improve with age, whereas others are severe and often associated with early demise and/or other organ involvement. This chapter introduces the clinical and histological characteristics and classifications of EBS. Subsequently, each protein that is defective in EBS is discussed, as are animal models of the disease.

2. Overview of epidermolysis bullosa simplex

Mutations in genes encoding keratinocyte components involved in the organization of the cytoskeleton or cell-cell junctions are responsible for EBS. EBS can be subclassified into basal and suprabasal according to the level of skin split [1, 2] **(Table 1)**.

Basal EBS is caused by defects in skin basement membrane (BMZ) proteins. **Figure 1** diagrams the skin BMZ. Among the BMZ components, keratin 5/14 and plectin are the main targets in EBS [3, 4]. A few EBS cases have been reported to have mutations in *ITGB4* and *COL17*, which encode β4 integrin and type XVII collagen, respectively [5, 6]. Recently, BPAG1-e was added to the list of basal EBS target proteins [7, 8].

	Subtype	Target gene (protein)
EBS	Suprabasal EBS	*PKP1* (plakophilin-1)
		DSP (desmoplakin)
		JUP (plakoglobin)
	Basal EBS	*KRT5* (keratin 5)
		KRT14 (keratin 14)
		PLEC (plectin)
		COL17 (type XVII collagen)
		ITGB4 (β4 integrin)

Table 1. Classification of EBS [1, 2]

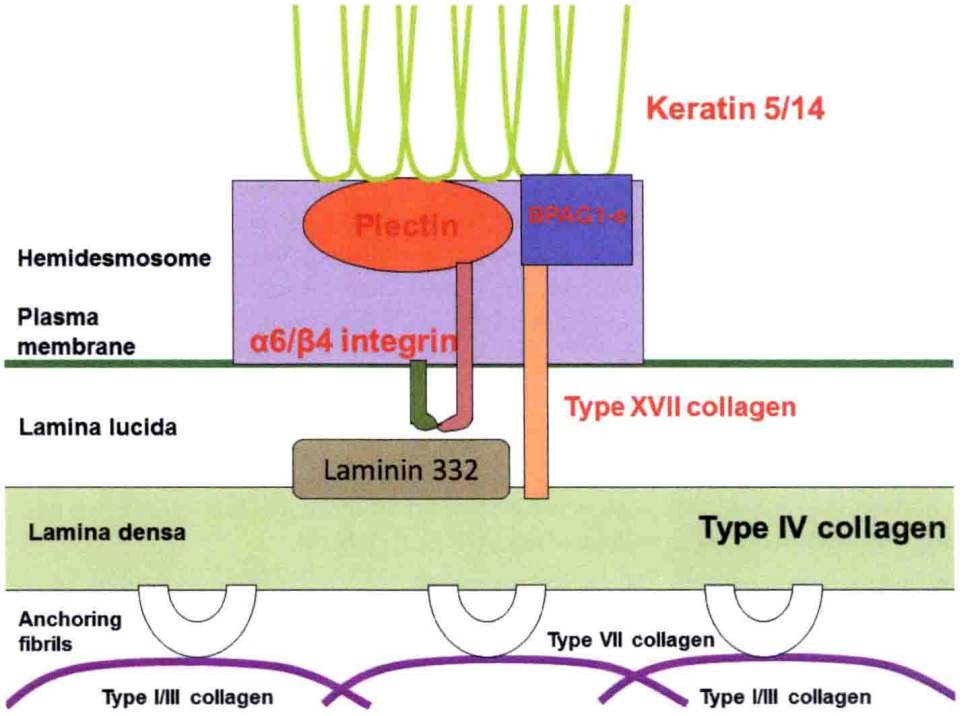

Figure 1. Schematic of the skin basement membrane zone. Components in red characters are target proteins of basal EBS.

In contrast, suprabasal EBS is associated with abnormalities in desmosomal proteins (**Figure 2**). So far, plakophilin-1, plakoglobin and desmoplakin are known to be the target proteins of suprabasal EBS [2, 9-11].

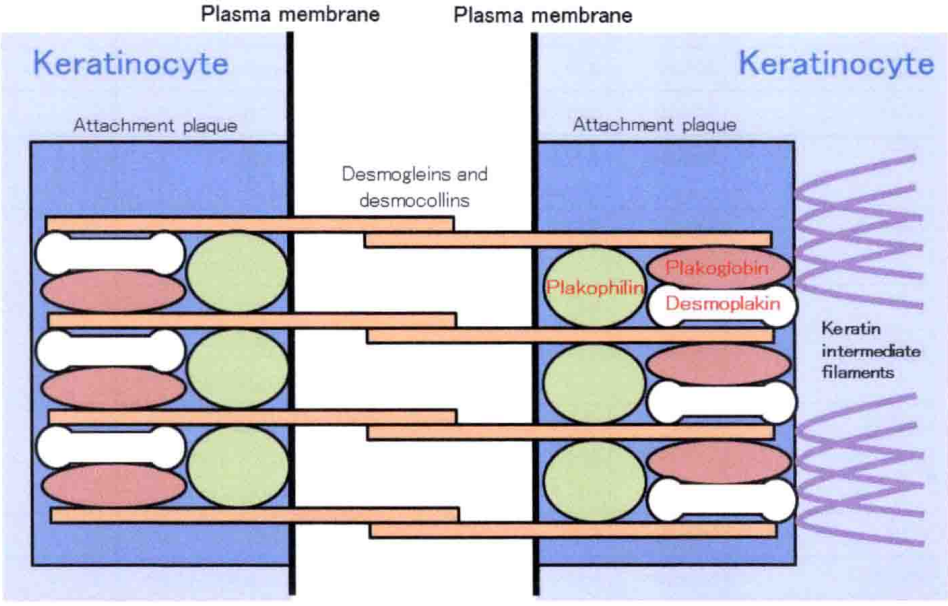

Figure 2. Schematic of desmosomes. Components in red characters are target proteins of suprabasal EBS.

Animal models have been used to clarify the function of some proteins and to develop new therapies for human diseases. Animal models of EB were reviewed recently [12, 13]. However, some new animal models have emerged since then [14, 15], and other transgenic mice with abnormalities in desmosomal proteins should be added to the list of EB animal models because of the introduction of the concept of "suprabasal EBS" [1]. **Table 2** summarizes animal models of EBS.

Causative Gene	Species	Type	Survival	Reference
KRT5	Mouse	KO	Neonatal death	[16]
KRT5	Cow	Naturally occurring (a heterozygous missense mutation)	Not mentioned	[17]
KRT14	Mouse	Tg (expressing truncated protein)	Neonatal death	[18]
KRT14	Mouse	KO	Neonatal death	[19]
KRT14	Mouse	KI	Neonatal death	[20]
KRT14	Mouse	KI (an inducible model)	Not mentioned	[20]
PLEC	Mouse	KO	Neonatal death	[21]
PLEC	Mouse	Conditional KO	Neonatal death	[22]
PLEC	Mouse	KI (expressing EBS-Ogna mutation)	Normal	[14]
DST	Mouse	KO	Not mentioned	[23]
DSP	Mouse	KO	Embryonicdeath	[24]
DSP	Mouse	Conditional KO	Not mentioned	[25]
PKP1	Dog	Naturally occurring (a homozygous splice donor site mutation)	Neonatal death (6 of 9 affected dogs)	[15]
JUP	Mouse	KO	Embryonicdeath	[26]
ITGB4	Mouse	KO	Neonatal death	[27]
ITGB4	Mouse	KO	Neonatal death	[28]
ITGB4	Mouse	Partial ablation (expressing ectodomain of β4 integrin)	Neonatal death	[29]
ITGB4	Mouse	Conditional KO	Not mentioned	[30]
COL17A1	Mouse	KO	Prolonged survival in 20% of mice	[31]

KO: knockout; Tg: transgenic; KI: knock-in

Table 2. Animal models of EBS [12-15]

3. Target proteins in basal EBS

3.1. Keratin 5/14

Recent brilliant reviews have addressed keratins and EBS [3, 32]. Here we focus on the history, mutation analysis, animal models and future therapeutics of keratin-associated EBS from the physician's point of view.

Keratin is one of the most abundant components of the epithelial cytoskeleton [33]. Typically, type I and type II keratins form heteropolymers that function in cells [34]. Keratin 5 (K5) and keratin 14 (K14) are specifically expressed in epidermal basal cells [34, 35] (**Figure 1**). In the 1980's, disorganization of those keratins was recognized in the basal keratinocytes of EBS patients [36, 37]. From those findings, it had been hypothesized that EBS patients have mutations in *KRT5* or *KRT14*, which encodes K5 or K14, respectively. In the early 1990's, transgenic mice overexpressing mutated K14 were reported to have severe skin fragility [18]. Soon after this discovery, two groups of researchers identified EBS cases with heterozygosity for *KRT14* missense mutations [38, 39], which were followed by the identification of the first EBS family with a heterozygous *KRT5* mutation [40]. Since then, several hundreds of EBS patients have been described as having *KRT5* or *KRT14* mutations and have been summarized in the Human Intermediate Filament Database (http://www.interfil.org/) [41].

There are several subtypes of keratin-associated EBS, as described in **Table 3** [1]. Classical and common EBS subtypes, in which traits are autosomal-dominantly inherited, are Dowling-Meara type EBS (EBS-DM), non Dowling-Meara type (EBS-gen-non-DM) and localized type (EBS-loc), from the severest to the mildest. Ultrastructurally, basal keratinocytes of EBS-DM are characterized by keratin aggregates [42]. Hot spots of the mutations in *KRT5* or *KRT14* are located within the helix-boundary motifs of each keratin [41]. A missense mutation in one allele of those regions (which leads to an amino acid alteration) typically exerts a dominant-negative effect on keratin organization. The severity of the clinical manifestations among EBS-DM, EBS-gen-non-DM and EBS-loc is generally determined by the site of the mutations and the difference between the original and the mutated amino acids [32]. However, it is not always easy to predict the phenotype from the underlying mutations and, in some cases, two different amino acid substitutions at the same codon result in different clinical manifestations [43, 44]. As a single amino-acid alteration does not necessarily cause a pathological change, *in vitro* and *in silico* systems to validate mutational effects have been proposed where keratin organization is visualized in cells transfected with mutated or wild-type keratins [44, 45].

The pathogenesis of EBS development through keratin mutations has also been demonstrated in animal models (**Table 2**). Following the discovery of transgenic mice overexpressing mutated K14 described above [18], *Krt5*-null and *Krt14*-null mice were reported to have a skin fragility phenotype [16, 19], although the condition of those mice was different from that of most EBS patients, where altered amino acids yield dominant-negative effects. Instead, those *Krt5*-null and *Krt14*-null mice show the phenotype of

autosomal recessive EBS (EBS-AR) whose K5 or K14 is null [32]. To reproduce dominant-negative effects of mutated keratins in human EBS (EBS-DM, EBS-gen-non-DM and EBS-loc), inducible knock-in EBS model mice were generated, in which a *Krt14* missense mutation equivalent to human EBS mutation was introduced [20]. This inducible EBS model recapitulates the skin fragility seen in human patients with autosomal dominant EBS. Furthermore, there is one naturally occurring bovine with a heterozygous *KRT5* mutation [17]. This Friesian-Jersey crossbred bull exhibits the EBS phenotype.

EBS, Dowling-Meara (EBS-DM)	Classical EBS
EBS, other generalized (EBS, gen-nonDM)	
EBS, localized (EBS-loc)	
EBS, autosomal recessive (EBS-AR)	
EBS with mottled pigmentation (EBS-MP)	
EBS, migratory circinate (EBS-Migr)	

Table 3. Keratin-associated EBS

Therapeutic interventions for EBS have been confined to palliative modalities. However, recent innovations in RNA interference have led to therapeutic strategies for dominant-negative disorders including keratin-associated EBS, where aberrant mutated keratin is knocked down while normal keratin synthesis on another allele is left intact [46]. This RNAi strategy is promising and will be further validated in clinical trials.

3.2. Plectin

A comprehensive review paper has addressed EBS and plectin [4], although there have been several advances in this field since then [14, 47-49].

Plectin is a cross-linking protein between the cytoskeleton and membranous proteins including hemidesmosomal components (**Figure 1**). Plectin has been known to have many transcript isoforms that differ from each other in N-terminal sequences at the protein level [50]. Among the many transcript isoforms, plectin 1a is the one that is mainly expressed in epidermal keratinocytes [51]. In addition to 5′ transcript complexity, plectin has a rodless splicing variant [52]. There are several EBS subtypes that are caused by plectin deficiencies (**Table 4**).

In the mid-1990's, mutations in the gene encoding plectin (*PLEC*) were discovered in patients with EBS with muscular dystrophy (EBS-MD) [53, 54]. Since then, many *PLEC* mutations, mostly located in the region encoding the rod domain of plectin, have been reported in EBS-MD patients [4, 47, 55].

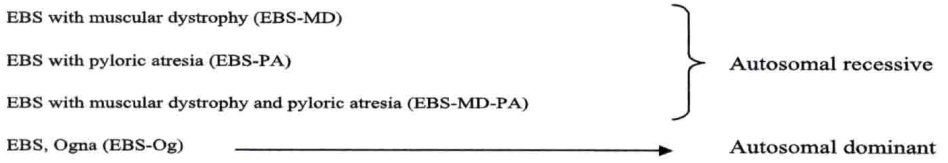

Table 4. Plectin-associated EBS

In 2005, two groups independently reported a new EBS subtype with *PLEC* mutations: EBS with pyloric atresia (EBS-PA) [56, 57]. EB with pyloric atresia (PA) had been known in patients with *ITGA6* or *ITGB4* mutations [58, 59]. However, skin specimens from those patients with integrin mutations show skin-split at the level of the lamina lucida, leading to the diagnosis of junctional EB (JEB). In contrast, EBS-PA cases with *PLEC* mutations were characterized by skin-split within epidermal basal cells [56].

The reason *PLEC* mutations lead to two distinct subtypes of EBS was clarified only recently. The development of monoclonal antibodies against several portions of plectin allowed us to understand the plectin expression patterns that distinguish between EBS-MD and EBS-PA [47]. EBS-MD skin typically shows the expression of rodless plectin without that of full-length plectin, whereas neither rodless nor full-length plectin is present in EBS-PA skin [47].

The next big question was whether EBS-MD and EBS-PA can occur simultaneously in a single patient or those two distinct EBS subtypes are mutually exclusive. Recently, one case was reported to have the phenotype of both EBS-MD and EBS-PA (EBS-MD-PA) [48]. The patient had truncation mutations at the last exon of *PLEC*, which resulted in the expression of diminished and shortened full-length and rodless plectin without the intermediate filament binding domain [48].

Apart from autosomal recessive EBS subtypes associated with *PLEC* mutations (EBS-MD, EBS-MD and EBS-MD-PA), there is one distinct autosomal dominant EBS with a *PLEC* mutation: EBS, Ogna (EBS-Og). EBS-Og is caused by a heterogeneous mutation of p. Arg2000Trp and is characterized by mild blister formation without MD or PA phenotype [4, 60]. To date, 5 unrelated families of EBS-Og have been reported to have the same mutation [49, 60].

Animal models of plectin-deficient EBS have been generated (**Table 2**). *Plec*-null mice show severe blistering phenotype and neonatal death [21], although gastrointestinal tracts were not investigated to confirm PA or PA-like lesions. Myofibril integrity is impaired in the skeletal and heart muscle of those mice [21]. Epidermis-specific ablation of plectin also elicits a severe blistering phenotype and early lethality in mice [22]. Furthermore, mice knocked-in with the murine equivalent mutation of EBS-Og show skin fragility due to epidermal-specific proteolysis of mutated plectin [14].

3.3. BPAG1-e

Dystonin, encoded by *DST*, has various isoforms in neural, muscle and epithelial tissue. BPAG1-e, also called BP230, is a major skin isoform of dystonin and a component of hemidesmosomes (**Figure 1**). BPAG1-e is known to be an autoantigen in bullous pemphigoid as well as type XVII collagen (C17) [61-63]. Since *COL17*, which encodes C17, was identified as a causative gene for non-Herlitz JEB [64], *DST*, which encodes BPAG1-e, had also been hypothesized for decades to be a target gene in other EB subtypes. However, it was only recently that mutations in *DST* were identified in autosomal recessive EBS patients [7, 8]. Those two patients typically had a mild acral blistering phenotype and had truncation mutations in the coiled-coil rod domain of BPAG1-e. Electron microscopy observation revealed loss of the inner plaque of hemidesmosomes in both cases [7, 8]. *Dst*-null mice show neural degeneration and mild skin fragility upon mechanical stress [23] (**Table 2**).

3.4. Miscellaneous

Mutations in *COL17* have been known to be responsible for non-Herlitz JEB (nH-JEB), in which the lamina lucida is the location of the skin-split as described above [64] (**Figure 1**). However, one case was reported to show a phenotype of EBS with *COL17* mutations [5]. The mutations found in that case caused a loss of intracellular C17 [5]. Furthermore, *Col17*-null mice were reported to show a reduced number of hypoplastic hemidesmosomal inner and outer attachment plaques with poor keratin filament attachment [31]. These findings suggest that *COL17* mutations can cause not only nH-JEB but also EBS, depending on the mutational sites.

α6/β4 integrins are hemidesmosomal components that are encoded by *ITGA6*/*ITGB4*, respectively. (**Figure 1**). Those genes are also target genes in JEB (with or without PA), just as *COL17* is a target gene in nH-JEB. There is one autosomal recessive EBS case where the intracellular portion of β4 integrin was deleted [6].

4. Target proteins in suprabasal EBS

4.1. Desmoplakin

Desmoplakin is a plakin family protein located in desmosome [55] (**Figure 2**). Two isoforms (desmoplakins I and II) are generated through alternative splicing [65]. Desmoplakin I is mainly expressed in the heart, whereas desmoplakin II is abundant in the skin [66]. In the early 1990's, desmoplakin was determined as a major autoantigen in paraneoplastic pemphigus [67, 68]. Mutations in the gene encoding desmoplakin, *DSP*, have been reported in several genodermatoses, mostly with cardiac manifestations [11, 69]. In 2005, a very severe EB case, referred to as lethal acantholytic epidermolysis bullosa (LAEB), was reported to have a homozygous deletion mutation in *DSP* [70]. The patient showed severe skin blistering and early demise. There have been only three reports on LAEB with *DSP* mutations [70-72]. Skin specimens in all the cases revealed acantholytic features in histopathology. From the correlation of clinical manifestations and mutational sites, it seems