Progress toward Treatment and Cure of Epidermolysis Bullosa: Summary of the DEBRA International Research Symposium EB2015

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Epidermolysis bullosa (EB), a group of complex heritable blistering diseases, is the topic of triennial research meetings organized by DEBRA International, Vienna, Austria, the network of national EB patient advocacy organizations. The DEBRA 2015 Research Conference, held in May 2015, brought together investigators and clinicians from around the world working at the forefront of EB research. Discussing the state-of-the-art approaches from a wide range of disciplines, there was a palpable excitement at this conference brought about by the optimism about applying new sequencing techniques, genome editing, protein replacement, autologous and allogeneic stem cell therapy, innovations in cancer biology, revertant mosaicism, and induced pluripotent stem cell techniques, all of which are aimed at developing new therapies for EB. Many in the field who have participated in EB research for many years were especially enthusiastic and felt that, possibly for the first time, the field seems uniquely poised to bring these new tools to effectively tackle EB. Multiple complementary approaches are currently in motion toward improved quality of life and eventually a cure for patients suffering from EB, a currently intractable disease.

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INTRODUCTION

Epidermolysis bullosa (EB), a group of heritable blistering disorders, consists of four main subtypes primarily distinguished by the level of blistering within the cutaneous basement membrane zone (Table 1). Each of these subtypes can display a spectrum of phenotypic severity reflecting the types and combinations of mutations in different genes, together with modifying environmental factors. The types of mutations also determine the mode of inheritance, either autosomal dominant or autosomal recessive. Currently 18 genes have been shown to be associated with the different subtypes of EB (Table 1). In spite of the tremendous progress made in understanding the molecular basis of different forms of EB, there is no cure for this disease.

DEBRA International, Vienna, Austria, an organization advocating on behalf of the patients with EB and their families, sponsors Triennial Research Conferences. The latest one in this series, organized by DEBRA of America, New York, in Braselton, GA, in May 2015, was attended by more than 100 researchers, physician-scientists, trainees, and patient support group representatives (Figure 1). This meeting report summarizes the presentations and discussions that took place in this conference.

MODEL SYSTEMS FOR EB

Animal models

In addition to many naturally occurring EB forms in animals reviewed previously (Bruckner-Tuderman et al., 2010, 2013; Uitto et al., 2010), a variety of model systems have been generated.

Novel murine models. Some recently developed animal models have revealed unexpected consequences and improved our understanding of phenotypic variability. For example, careful analysis of mouse models for junctional EB (JEB) identified the first major genetic modifier of JEB phenotype due to a laminin-γ2 mutation by collagen XVII, in particular molecular variations in its NC4 domain (Sproule et al., 2014). Also, a recently reported knock-in mouse model for JEB that displays alternative splicing of the *Lamb3* gene will aid in defining further genetic modifiers of JEB phenotypes (Hammersen et al., 2015).

Another interesting finding relating to junctional skin blistering was revealed by the deletion of the linker extracellular domain of transmembrane collagen XVII in mice. This led to alternative shedding of the ectodomain, but not to JEB. Instead, induction of autoimmune blistering and itching were observed, and the phenotype of the mice mirrored signs of bullous pemphigoid, including perilesional eosinophilic infiltrations, blood eosinophilia, and elevated serum IgE levels (Hurskainen et al., 2015). Future work will be aimed at discerning mutations and disease mechanisms predisposing to mechanobullous versus inflammatory blistering phenotypes in both humans and mice.

Because of the multiorgan involvement, the severity of the phenotypes, and significant unmet medical need, the

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Abbreviations: BM, bone marrow; BMT, bone marrow transplantation; DEB, dystrophic EB; EB, epidermolysis bullosa; HMGB1, high mobility group box 1; iPSCs, inducible pluripotent stem cells; JEB, junctional EB; MSC, mesenchymal stromal/stem cell; RDEB, recessive DEB; SCC, squamous cell carcinoma

Table 1. Molecular heterogeneity of different forms of EB

Disease	Gene	Cytogenetic location	Inheritance	Proportion of EB attributed to mutations in this gene
Simplex epidermolysis bullosa (EBS)	*KRT5*	12q13.13	AD	75% of EBS-AD cases;
	KRT14	17q21.2	AR, AD	15 cases of EBS-AR have been reported with KRT14 mutations
	TGM5	15q15.2	AR	Up to 10% cases of EBS
	DSP	6p24.3	AR	
	PKP1	1q32.1	AR	
	JUP	17q21.2	AR, AD	
	EXPH5	11q11.3	AR	
	PLEC	8q24.3	AR, AD	
	DST	6p12.1	AR	
	ITGB4	17q25.1	AR	
	COL17A1	10q24.3-q25.1	AR	
Junctional epidermolysis bullosa (JEB)	*LAMA3*	18q11.2	AR	9% of all JEB cases; specific mutations in the LOC (Shabir) syndrome
	LAMB3	1q32.2	AR	70% of all JEB cases
	LAMC2	1q25.3	AR	9% of all JEB cases
	COL17A1	10q24.3-q25.1	AR	10% of all JEB cases
	ITGA6	2q31.1	AR	A few cases reported
	ITGB4	17q25.1	AR	Many cases reported
	ITGA3	17q21.33	AR	A few cases reported
Dystrophic epidermolysis bullosa (DEB)	*COL7A1*	3p21.31	AR, AD	100% of all DEB cases
Kindler syndrome (KS)	*FERMT1*	20p12.3	AR	100% of all KS cases

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; LOC, laryngo-onycho-cutaneous syndrome.

dystrophic forms of EB (DEB) have been the focus of many investigations often using previously developed collagen VII knockout or hypomorphic mice (Fritsch et al., 2008; Heinonen et al., 1999). In addition, a rat model for dominant DEB, which exhibits a gene dosage effect, offers a possibility of evaluating the influence of modifier genes on DEB phenotype (Nyström et al., 2013).

Zebrafish and drosophila. Interesting alternative animal models to study EB have recently been reported, including zebrafish and *drosophila*. Several of the EB-relevant genes are expressed in zebrafish, and therefore, this model system has been used to generate skin-blistering phenotypes reflecting features of EB, such as morpholino-mediated knockdown of collagen XVII gene expression (Kim et al., 2010; Li and Uitto, 2014). Recent work has used the keratin-free tissue environment in *drosophila* to investigate the formation of keratin networks and to define mechanisms by which mutated keratins cause cellular pathology (Bohnekamp et al., 2015). Human keratins 5 and 14, when expressed in *drosophila* epithelia, formed well-organized keratin networks thus validating the fly as a novel genetic model system for keratin physiology and pathology. Inclusion of a mutated keratin 14 in the networks caused semilethality, wing blisters, and perturbed cellular integrity. This *drosophila* model of EBS will be valuable for further investigation of the effects of different keratin mutations, their cellular consequences, and possibilities for therapeutic interventions.

Organotypic cultures

Yet another model to investigate disease mechanisms and test therapeutic approaches is the 3D skin equivalent organotypic cultures. One study treated grafted human recessive DEB (RDEB) equivalents topically with recombinant human collagen VII and showed that the therapeutic collagen restored

Figure 1. Participants in the EB2015 Research Symposium held in Braselton, GA, in May 2015.