Expression of caspase 8 in lesional and non-lesional skin of epidermolysis bullosa simplex patients

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Masters Degree in Dermatology

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بسم الله الرحمن الرحيم

{وما توفيقي إلا بالله عليه توكلت وإليه أنيب}

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Abstract

Background: Epidermolysis bullosa simplex (EBS) is the most common type of EB, accounting for 70% of cases. Mutations in keratin 5 and keratin 14 genes are responsible for the four major types of EBS. The susceptibility of keratinocytes to caspase-8-mediated apoptosis is suggested to be increased in mutated K14 keratinocytes.

Aim of the work: The current study aims at studying the expression of caspase 8 in lesional and non-lesional skin of EBS patients in comparison to normal control skin in order to highlight the possible role of apoptotic/inflammatory pathways in the pathogenesis of this disease.

Subjects and methods: This cross sectional case control study was conducted on 10 patients proved to have EBS by electron microscopic examination. They underwent two 4 mm punch biopsies; one from a fresh blister and the other from non-lesional skin, for histopathological assessment of the density of dermal infiltrate and for immunohistochemical detection of caspase 8. Five age and sex matched healthy volunteers served as controls.

Results: Caspase 8 expression both in lesional and non-lesional skin was significantly higher than in controls ($p<0.01$ and $p=0.013$ respectively). No significant difference existed as regards caspase 8 expression between lesional and non-lesional skin ($p=1$). The density of dermal infiltrate was significantly higher in lesional skin *(p=0.02).* A strong significant positive correlation was found when correlating caspase 8 expression in lesional skin with the extent of the disease, the rate of blistering, and the density of dermal infiltrate (*r=0.835, p=0.003, r=0.889, p=0.001and r= 0.776, p=0.008* respectively).

Conclusion: Keratin mutation is only a conductor of an orchestra of events in the pathogenesis of EBS. Caspase-8-mediated apoptosis is an integral component of this orchestra. We propose the term apo-cytolysis to better describe the exact mechanism of blistering in EBS. Inflammation is secondary to apo-cytolysis.

Keywords: Epidermolysis bullosa simplex, caspase 8, apoptosis, inflammation.

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List of abbreviations

AD	Autosomal dominant
ADP	Adenosine diphosphate
AF	Anchoring fibrils
AIF	Apoptosis inducing factor
ALPS	Autoimmune lymphoproliferative syndrome
Apaf-1	Apoptotic protease activating factor 1
APO	Apoptosis antigen
AR	Autosomal recessive
Arg	Arginine
BAK	Bcl-2 homologous antagonist/killer
BAX	Bcl-2-associated X protein
Bcl2	B-cell lymphoma 2
Bcl-XL	B-cell lymphoma-extra large
BDN	Bullous dermolysis of the newborn
BH3	Bcl-2 homology domain 3
Bid	BH3 interacting-domain death agonist
BMZ	Basement membrane zone
BP	Bullous pemphigoid
BPAG1-e	Epithelial isoform of bullous pemphigoid antigen 1
CAD	Caspase activated DNAse
CD	Cluster of differentiation
COL17A1	Type XVII collagen alpha-1 gene
COL7A1	Type VII collagen alpha-1 gene
Cys	Cysteine
CytoC	Cytochrome C
Daxx	Death-associated protein 6
DD	Death domain
DEB	Dystrophic epidermolysis bullosa
DDEB	Dominant DEB
DDEB-ac	DDEB-acral
DDEB-BDN	DDEB-bullous dermolysis of the newborn
DDEB-gen	DDEB-generalized
DDEB-na	DDEB-nails only
DDEB-pr	DDEB-pruriginosa
DDEB-pt	DDEB-pretibial
DED	Death effector domain
DEJ	Dermo-epidermal junction
Del	Deletion
DEXA	Dual-emission x-ray absorptiometry
DFF	DNA fragmentation factor
Diablo	Direct IAP binding protein with low pI
DISC	Death inducing signaling complex
DNA	Deoxyribonucleic acid
DR	Death receptor
DSP	Desmoplakin

DST	Dystonin
EB	Epidermolysis bullosa
EBS	Epidermolysis bullosa simplex
EBS-AR	EBS-autosomal recessive
EBS-DM	EBS Dowling Meara
EBS-gen-nDM	EBS generalized non Dowling Meara
EBS-loc	EBS localised
EBS-MD	EBS with muscular dystrophy
EBS-Migr	EBS migratory circinate
EBS-MP	EBS with mottled pigmentation
EBS-Og	EBS Ogna
EBS-PA	EBS with pyloric atresia
EBSS	EBS superficialis
EDAR	Ectodysplasin A receptor
EGF	Epidermal growth factor
EM	Electron microscopy
EndoG	Endonuclease G
ERK	Extracellular signal-regulated kinase
FADD	Fas Associated via Death Domain
FasL	Fas ligand
FERMT1	Fermitin family homologue-1 gene
FLICE	FADD-like interleukin-1 beta-converting enzyme
FLIP	FLICE inhibitory protein
GABEB	Generalized atrophic benign EB
GMP	Good manufacturing practice
HD	Hemidesmosome
HTRA2	High temperature requirement protein-2
IAPs	Inhibitor of apoptosis proteins
IFM	Immunofluorescence mapping
In-frame del/ins	In-frame deletion and insertion
Ins	Insertion
ITGA6	Integrin α6
ITGB4	Integrin ß4
JEB	Junctional epidermolysis bullosa
JEB-H	JEB-Herlitz
JEB-I	JEB-inversa
JEB-Lo	JEB-late onset
JEB-nH	JEB-non-Herlitz
JEB-nH gen	JEB-non Herlitz generalized
JEB-nHloc	JEB-non Herlitz localized
JEB-O	JEB-other
JEB-PA	JEB with pyloric atresia
K	Keratin
kDa	Kilo Dalton
KIF	Keratin intermediate filaments
KIND1	Kindilin-1 gene
KRT	Keratin
KS	Kindler syndrome
LAMA3	Laminin alpha-3 gene

LAMB3	Laminin beta-3 gene
LAMC2	Laminin gamma-2 gene
LOC	laryngo-onycho-cutaneous syndrome
LPS	Lipopolysaccharide
MS	Missense mutation
NC 1	the amino terminal non-collagenous domain
NFJS	Naegeli–Franceschetti–Jadassohn syndrome
NF-^{k}B	Nuclear factor ^{k} B
NS	Nonsense mutation
PAS	Periodic Acid Schiff
PKP1	Plakophilin-1
PLEC1	Plectin
Ras	Rat sarcoma, the most common oncogene in human cancer.
RDEB	Recessive DEB
RDEB-BDN	RDEB-bullous dermolysis of the newborn
RDEB-Ce	RDEB-centripetalis
RDEB-GS	RDEB-generalized severe
RDEB-O	RDEB-generalized other
RDEB-pr	RDEB-pruriginosa
RDEB-pt	RDEB-pretibial
RDEB-sev/gen	Severe generalized RDEB
RIP1	Receptor-interacting protein-1
RNA	Ribonucleic acid
SBDP	Sub-basal dense plate
SCC	Squamous cell carcinoma
SMAC	Second mitochondria-derived activator of caspase
Spl	Splice site mutation
tBid	Truncated/cleaved Bid
TcR	T cell receptor
TEM	Transmission electron microscopy
TNF	Tumor necrosis factor
TNFR	TNF receptor
TRADD	TNF receptor-1-associated death domain
TRAF2	TNF receptor associated factor-2
TRAILR	TNF-related apoptosis-inducing ligand receptor
UV	Ultraviolet

Introduction

Epidermolysis bullosa (EB) is a group of inherited blistering diseases, which appear at birth, or shortly thereafter, and are characterized by trauma-induced blister formation ***(Sawamura et al., 2010).***

EB is caused by mutations within genes that encode structural proteins that reside within the epidermis (EB simplex), dermo-epidermal junction (junctional EB), or uppermost papillary dermis (dystrophic EB). The site within which each of these proteins resides determines the ultrastructural location where the blisters arise. EB simplex is the most common type of EB, accounting for 70% of cases ***(Fine et al., 2008).***

Mutations in keratin 5 and keratin 14 genes are responsible for the four major types of epidermolysis bullosa simplex ***(Irvine, 2005).***

The susceptibility of keratinocytes to caspase-8-mediated apoptosis is suggested to be increased in mutated K14 keratinocytes ***(Yoneda et al., 2004).*** Caspase 8 plays an essential role in the execution of death receptor-mediated apoptosis ***(Ashkenazi, 2002).*** Whether the mechanisms underlying the pathogenesis of EBS are purely genetic or contributed also by apoptotic inflammatory responses remains to be a puzzling issue ***(Kerns et al., 2007).***

Increasing evidence suggests that caspases have important functions to regulate cell proliferation, differentiation and migration in addition to apoptosis regulation ***(Acehan et al., 2002).***

Aim of the work

The current study aims at studying the expression of caspase 8 in lesional and non-lesional skin of EBS patients in comparison to normal control skin in order to highlight the possible role of apoptotic/inflammatory pathways in the pathogenesis of this disease.

Chapter 1

Epidermolysis Bullosa, an overview with special emphasis on epidermolysis bullosa simplex

Introduction:

Epidermolysis bullosa (EB) comprises a group of genetically determined disorders characterized by skin fragility, blistering of the skin and mucosae following mild mechanical trauma. Therefore, the alternative term is *mechanobullous diseases*. The descriptive term *epidermolysis* is illogical because epidermal disruption is not the primary change in two of three main categories of EB. However, the name *epidermolysis bullosa*, as originally used by ***Koebner*** in ***1886,*** is so well established in the literature that it is still the preferred term ***(Pearson, 1962).***

Classification:

Classification of this complex and heterogeneous group of disorders is difficult and not helped by the large variety of names and eponyms that have traditionally been used. Early classification was based largely on the mode of inheritance and clinical studies involving relatively few patients and families ***(Cockayne, 1993).***

While these early observations were clearly important in establishing EB as an entity, a major step forward was made by ***Pearson*** in ***1962***, who used electron microscopy to show that the ultrastructural level of tissue cleavage *i.e.* blister formation in the skin is distinctive in the three major groups of EB: EB simplex, junctional EB and dystrophic EB ***(Eady et al., 2004).***

The last consensus classification contains an extensive description of the various EB subtypes. Recent changes to the classification scheme have included Kindler syndrome as the fourth major subtype of EB ***(Fine et al, 2008)***.

I. EB Simplex:

EBS is the most frequent form of EB, accounting for at least 70% of patients ***(Fine et al., 2008).*** EBS is the most superficial type of EB, with blisters arising intraepidermally in response to trauma **(Fig. 1)**.

Most forms of EB simplex (EBS) are transmitted in an autosomal dominant manner. There are two main subgroups of EBS, suprabasal and basal, which differ in the ultrastructural level of the intraepidermal blistering .The vast majority of EBS cases are in the basal group, most often resulting from a dominant-negative mutation within the keratin 5 (*K5*) or 14 (*K14*) genes, expression of which is primarily within the basal layer of the epidermis. Suprabasal forms of EBS are caused by mutations in the genes encoding the desmosomal proteins plakophilin-1, plakoglobin and desmoplakin ***(Fuchs, 1999).***

An autosomal recessive form of EBS due to mutations in the gene encoding plectin is associated with muscular dystrophy (EBS-MD), which is not surprising considering that this protein is expressed in skeletal muscle as well as in the hemidesmosomes of basilar keratinocytes; other patients with plectin deficiency present with pyloric atresia (EBS-PA) ***(Shimizu et al., 1999).***