Pathology Elsewhere

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The role of collagen XVII in regulating keratinocyte migration

Acquired or inherited junctional epidermolysis bullosa (JEB) are characterized by a separation between the epidermis and the dermis, causing blistering skin disorders. Mutations in the COL17A1 gene, coding for BPAG2 (bullous pemphigoid antigen 2) or in collagen XVII, expressed by epithelial cells and forming hemidesmosomes, result in a clinically less severe form, the nonlethal type of JEB (nJEB). This finding suggests that collagen XVII participates in the adhesion of basal keratinocytes to the extracellular matrix. An association of nJEB and laminin-5 mutations has also been reported. Laminin 5 is a heterotrimer protein composed of three subunits, $\alpha 3$, $\beta 3$, and $\gamma 2$, which are encoded by the genes LAMA3, LAMB3, and LAMC2, respectively. Laminin 5 is the extracellular ligand of $\alpha 6\beta 4$ integrin in hemidesmosomes. By studying the behavior of keratinocytes with null mutations in the COL17A1 gene, **Tasanen *et al***¹ observed that initial adhesion of mutant cells to laminin 5 was not affected, but the spreading of these cells was enhanced. BPAG2- or collagen XVII-deficient keratinocytes were also capable of synthesizing and processing laminin 5, but the laminin 5 deposits were scattered and poorly organized. This finding suggests that correct integration of laminin 5 within the matrix also requires collagen XVII. This assumption was supported by the codistribution of BPAG2 and laminin 5 in the extracellular matrix around normal keratinocytes, mediated by direct interaction with laminin through its C-terminus. Absence of BPAG2 weakened the anchorage of basal keratinocytes to the basement membrane and blisters developed despite the presence of laminin 5. A variety of mutations of laminin 5 subunit genes led either to absence of laminin 5 in the basement membrane or expression of structurally altered laminin 5 in nJEB. The lack of laminin 5 interactions with both integrin and collagen XVII may explain the more severe phenotype in patients lacking laminin 5. Thus, this paper provides a detailed structural understanding of protein:protein interactions in the hemidesmosomal apparatus of keratinocytes in bullous pemphigoid.

In this issue of *Laboratory Investigation*, **Buchroithner *et al***² have identified a novel abnormality in a patient with nJEB: an allelic nonsense mutation in combination with an allelic mutation that create an exonic splice site in *LAMB3* gene, which encodes for the $\beta 3$ polypeptide chain of

laminin 5. The allelic mutation-induced splice site was preferentially used over the wild-type splice site, which goes against the general rules of pre-mRNA splicing and RNA degradation. Both studies underscore the importance of collagen XVII and laminin in anchoring keratinocytes to the basement membrane. Furthermore, they demonstrate the insights that can be gained from genetic analysis of JEB, and may provide opportunity for mutation-specific gene therapy.

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References

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Different Smad, different cell type, different effect on TGF- β signaling

Smad transcription factors mediate the growth inhibitory effect of transforming growth factor- β (TGF- β) in many cell types. The name Smad was derived from a contraction of *Drosophila* (Mad) and *Caenorhabditis elegans* (Sma) where these transcription factors were first identified. Following ligand binding at the cell surface, receptors for TGF- β form a heterodimeric complex that phosphorylates the receptor-activated Smads (Smad 2 and Smad 3). These then associate with Smad 4 and translocate to the nucleus. The Smad proteins can then activate or repress transcription of TGF- β -dependent target genes. Deregulation of TGF- β signaling, which often involves Smads, is usually seen in tumor or pathologic fibrosis. While both Smads 2 and 3 mediate signals from TGF- β , these two Smads clearly possess nonredundant functions. While Smad3 binds DNA directly, Smad2 instead activates transcription indirectly by binding to transcription factors.

A published study by **Kretschmer *et al***¹ compared the inhibitory effect of Smad 2, Smad 3 and Smad 4 side-by-side in differentiation-competent cells and analyzed the resulting effects on the endogenous TGF- β signal transduction with respect to gene expression and growth inhibition. The study was accomplished by selectively suppressing protein expression using Gene Blocs antisense molecules against each individual Smad molecule. In HaCaT

keratinocytes, Smad 3 was the predominant mediator of TGF- β -induced growth inhibition. Inhibition of Smad 3 expression was sufficient to interfere with TGF- β -induced cell cycle arrest and to induce or suppress endogenous cell cycle regulators (modulating expression of c-Myc and p21, and the phosphorylation status of Rb). Inhibition of Smad 4 expression exhibited a partial effect, whereas inhibition of Smad 2 expression had no effect in keratinocytes. In MCF-10A breast epithelial cells, Smad 2, Smad 3 and Smad 4 contributed equally to TGF- β signaling. These findings indicated that different Smad complexes may be responsible for the regulation of different genes within one cell type and emphasized the contribution of individual Smads to TGF- β signaling with respect to their tumor suppressive role.

In this issue, **Saika *et al*²** show that Smad 3 function is critical to the development of proliferative vitreoretinopathy (PVR) following retinal detachment. PVR is the result of production of α -smooth muscle actin and pathogenic accumula-

tion of extracellular matrix proteins, which ultimately contributed to traction detachment of the retina.

Both studies demonstrate the importance of Smad signalling in the development of fibrosis. Elucidating the potential role of Smad proteins in diseases with deregulated TGF- β signaling such as tumor, brosis, defects in wound healing or immune responses will aid in the development of more specific approaches for therapeutic intervention.

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References

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