Messages from Mutant Desmosomes

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Single gene disorders are ideally suited to establish robust genotype–phenotype correlations and provide excellent opportunities to understand molecular pathomechanisms with relevance to complex disorders. The observation that patients diagnosed with the same causative mutation can present with phenotypic disease variability illustrates the significant role of disease modifiers and warns against oversimplification. In a new article in the *Journal of Investigative Dermatology*, Zimmer et al. (2021) analyze two mutations located in the desmoglein (DSG) 1 transmembrane domain (TMD) and find that both mutants fail to assemble into desmosomes owing to reduced membrane trafficking and lipid raft targeting. One mutation maintained normal protein expression levels and turnover relative to those of wild-type (WT) DSG1, and behaved as a dominant negative. The second mutant showed reduced stability and increased turnover compared with WT DSG1 as well as reduced desmosome size and abundance. A full understanding of the TMD of DSG1 requires cell biological approaches, underscoring the value of cell biology in biomedical research in general.

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Desmosomes are calcium-dependent, intercellular adhesive complexes found in epithelia, endothelia, heart muscle, and the meninges surrounding the brain. They connect the intermediate filament (IF) cytoskeleton to the plasma membrane (Simpson et al., 2011) and are particularly abundant in tissues exposed to mechanical stress, including skin and the heart. Desmosomes comprise three main protein families: the transmembrane cadherins (desmogleins [DSGs] 1–4 and desmocollins [DSCs] 1–3), armadillo proteins (PKP 1–3 and JUP), and plakin proteins (DSP). DSGs and DSCs of neighboring cells form trans-interactions that mediate strong cell–cell adhesion. The cytoplasmic domains of DSGs and DSCs interact with armadillo proteins, which directly bind to DSP, a major IF linker protein. In stratified epithelia, such as the mammalian epidermis, desmosome composition varies with differentiation, such that DSG1 is predominantly expressed in superficial desmosomes, whereas DSG3 predominates in proliferating basal keratinocytes (Simpson et al., 2011).

Autoantibodies, bacterial toxins, and mutations can compromise desmosome function and lead to tissue-specific disorders that can be life threatening (Broussard et al., 2015; Samuelov and Sprecher, 2015). Monoallelic mutations in the *DSG1* gene cause striate palmoplantar keratoderma (PPK), a focal PPK characterized by linear hyperkeratosis along the flexors of the fingers and on the palms as well as focal hyperkeratosis of the plantar skin (Online Mendelian Inheritance in Man [OMIM] #148700). In contrast, biallelic *DSG1* mutations give rise to severe dermatitis, multiple allergies, metabolic wasting (SAM) syndrome, characterized by congenital erythroderma, dermatitis, ichthyosis, severe PPK, palmoplantar keratosis, multiple food allergies, and metabolic wasting (OMIM #615508). The difference in severity between PPK and SAM syndrome may result from several causes, including gene dosage. PPK arises from *DSG1* haploinsufficiency, whereas the entire loss of functional *DSG1* in SAM syndrome results in more severe disease. In addition, the signaling functions of *DSG1* may be compromised in specific ways. Why SAM syndrome manifestations vary from mild to very severe despite complete *DSG1* deficiency in all cases remains incompletely understood.

To address this variability, Zimmer et al. (2021) compared two mutations located just five amino acid residues apart in the DSG1 transmembrane domain (TMD). A glycine (Gly) residue was changed to an arginine (Arg) residue in both, with G557R causing PPK and G562R resulting in SAM syndrome. Along with wild-type (WT) *DSG1*, the two disease-associated mutations ($DSG1_{PPK–TMD}$ and $DSG1_{SAM–TMD}$) were expressed as GFP-tagged variants in A431 cells, an immortal human epidermal carcinoma cell line that lacks endogenous DSG1 and is widely used in studies of desmosomes. The chosen strategy phenocopied the setting of SAM syndrome but not that of PPK where a mutant and a WT allele coexist.

Immunofluorescence and western blotting documented a reduced expression of the $DSG1_{PPK–TMD}$ GFP mutant relative to that of $DSG1_{WT}$ GFP or $DSG1_{SAM–TMD}$ GFP. Colocalization with endogenous DSP revealed that both Gly:Arg substitutions in the $DSG1_{TMD}$ compromised the ability of these mutants to support normal desmosome formation. Consistent with the smaller size and reduced number of desmosomes, both mutants showed diminished adhesion in an epithelial sheet assay (Huen et al., 2002) compared with WT DSG1, with $DSG1_{SAM–TMD}$ GFP being inferior to $DSG1_{PPK–TMD}$ GFP. Relative resistance to detergent extraction can serve as a readout for keratin-attached and fully assembled desmosomes (Pasdar and Nelson, 1989). Using such conditions, both mutations exhibited increased solubility compared with $DSG1_{WT}$ GFP. It has been recently shown that the length of a protein TMD is a determinant of raft partitioning, with longer TMDs preferring more ordered domains

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COMMENTARY

Clinical Implications

- Even closely related mutations in desmosomal components can give rise to distinctly different diseases.
- Understanding desmoglein (DSG) 1 trafficking, turnover and protein interactions is a prerequisite for the correct diagnosis and treatment of desmosomal disorders.
- Insights from some cystic fibrosis treatments might be relevant for DSG-related disorders.

(Lorent et al., 2017). Given the raft association of desmosomes, the mutant DSG1 TMDs were probed using a structure prediction algorithm. This indicated that the introduction of an Arg residue breaks the α-helix, shortening the TMD from 24 residues in WT to 11 residues in $DSG1_{PPK}$ TMD and 16 residues for $DSG1_{SAM}$ TMD. Notably, TMD length serves as a determinant of subcellular localization and raft association of transmembrane proteins. Proteins with short domains localize to the endoplasmic reticulum and Golgi, whereas those with longer ones localize to the cell membrane and can associate with rafts (Diaz-Rohrer et al., 2014). Because protein palmitoylation is a clear raft-targeting signal and palmitoylation of DSG1 occurs at Cys570/571/573, it would be interesting to determine whether these disease mutations prevent palmitoylation. To probe for raft association of both mutants, predictably affected by these physical features, density gradient fractionations to isolate detergent-resistant, raft membranes (DRMs) from nondetergent, nonraft membranes (non-DRMs) were performed. Results pointed to defective raft targeting of $DSG1_{TMD}$ mutants and the reduction of JUP in desmosomal raft domains as a cause for the reduced number, size, and adhesive strength of such desmosomes. Although both $DSG1_{TMD}$ mutants exhibited delayed trafficking through the biosynthetic pathway to the cell surface, they differed in their steady-state surface levels, with $DSG1_{PPK–TMD}$ GFP being considerably less stable than $DSG1_{SAM–TMD}$ GFP. To address the impact of both mutations on protein stability directly, lysosomal and proteasomal degradation pathways, both involved in the clearance of desmosome components, were assayed on inhibition of protein biosynthesis. Compared with $DSG1_{WT}$ GFP and $DSG1_{SAM–TMD}$ GFP, $DSG1_{PPK–TMD}$ GFP showed increased protein turnover, which likely explains its lower abundance.

Thus, the $DSG1_{SAM–TMD}$ GFP mutant is expressed at the cell surface and accumulates at steady-state levels similar to $DSG1_{WT}$ GFP, whereas the $DSG1_{PPK–TMD}$ GFP mutant exhibits rapid lysosomal and proteasomal degradation. However, $DSG1_{PPK–TMD}$ mutants that reach the cell surface appear unstable and undergo enhanced endocytosis, followed by lysosomal degradation. $DSG1_{SAM–TMD}$ GFP, in contrast, largely escapes degradation and arrives at the plasma membrane where it compromises desmosome function and adhesive strength in a dominant-negative manner, possibly by accumulation as an extradesmosomal component.

The findings of Zimmer et al. (2021) raise the question of how the occurrence of Arg substitution mutations located close to each other causes such different tissue pathology and clinical features. The authors note that a key biochemical difference between both mutants involves protein stability. This matters most in the palmoplantar epidermis in which desmosome protein levels and size of desmosomes are increased in healthy but not in $DSG1_{PPK–TMD}$ individuals. The continuous wear and tear at these sites, in combination with smaller and fewer desmosomes, can explain a haploinsufficient disease mechanism. Mechanical stress as a disease modifier is consistent with the role of DSC2 and DSP in mechanosensing (Baddam et al., 2018; Price et al., 2018).

In addition to their mechanical function, desmosomal proteins, including DSG1, are involved in signaling pathways and protein interaction networks. A second, not entirely separate mechanism underlying SAM syndrome and PPK relates to the involvement of DSG1 in promoting epidermal differentiation by dampening RAS/MAPK signaling intensity (Harmon et al., 2013). Elevated RAS/MAPK activity has been reported in the skin of patients with PPK (Simpson et al., 2011), and PPK symptoms have been observed in individuals diagnosed with RASopathies (Hammers and Stanley, 2013). A third mechanism that might explain the distinctly different features of SAM syndrome and PPK may emerge from recent work substantiating a link between DSG1 and the gap junction transmembrane protein Cx43, mutations of which cause erythrokeratodermia variabilis (OMIM #133200), a skin condition with some features common to those described in this paper. Although the exact mechanisms by which *Cx43* mutations affect epidermal differentiation and barrier function remain uncharacterized, a decrease of *Cx43*- in *DSG1*-mutant SAM syndrome biopsy samples and cultured cells has been reported (Cohen-Barak et al., 2020). In view of the emerging proteome of *Cx43* and of desmosomal components (Badu-Nkansah and Lechler, 2020), a large number of protein candidates have emerged that lend themselves to future mechanistic studies to unravel the overlapping and unique functions of *DSG1* mutants. It is conceivable that extradesmosomal *DSG1* undergoes novel protein interactions, which ultimately might contribute to inflammatory conditions typical of SAM syndrome (Figure 1).

The trafficking defects of *DSG1* mutants studied by Zimmer et al. (2021) are reminiscent of those in the CFTR, caused by numerous mutations. In-depth studies of CFTR trafficking mutants, many of which accumulate in intracellular compartments, have ultimately led to the development of drugs that promote CFTR surface localization and improve the disease (Gramegna et al., 2021). With increasing insight into the cell biology of desmosomes, options to treat rare but life-threatening desmosomal disorders may become available in the not-so-distant future.

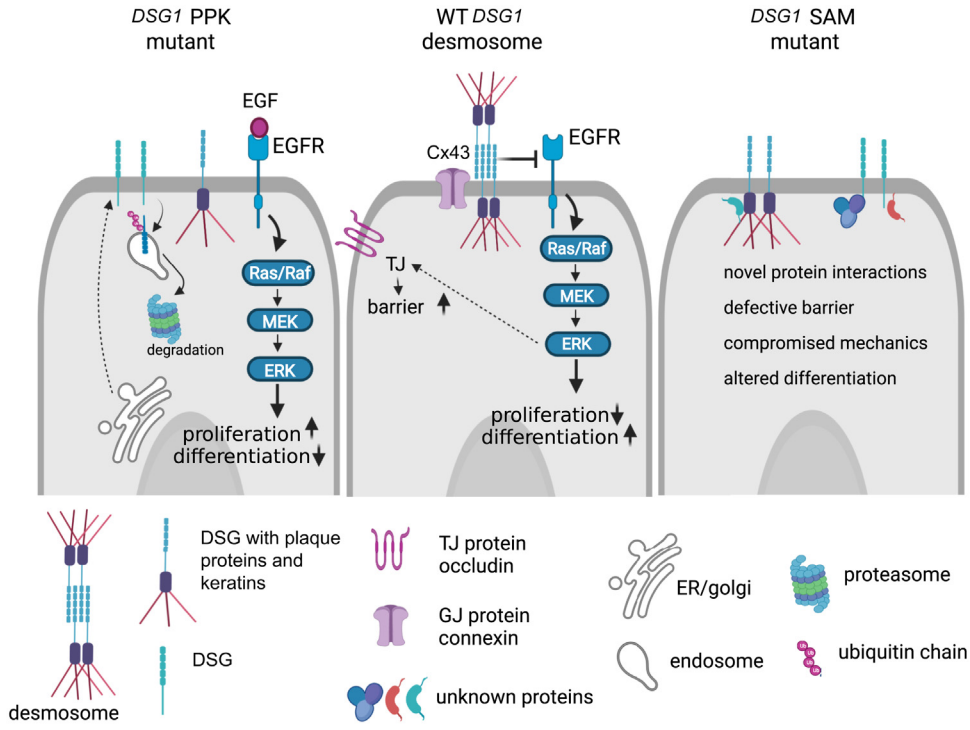

Figure 1. Different pathomechanisms result from two unique mutations located in the transmembrane domain of DSG1. Middle: WT desmosomes (desmosomal cadherins, blue; plaque proteins, dark blue; keratins, violet) maintain strong intercellular adhesion, serve as mechanosensors, and control differentiation and barrier formation by EGFR (light blue) signaling that also controls epidermal barrier function through TJs. Furthermore, desmosomes network with the GJ protein Cx43. Left: the PPK-associated $DSG1_{G557R}$ mutant (blue) shows elevated turnover and reduced appearance at the cell surface from where it is endocytosed and degraded. As a result, fewer desmosomes remain, which may be insufficient to sustain palmoplantar tissue integrity under strain. Right: the SAM syndrome–associated $DSG1_{G562R}$ mutant (blue) showed slower traffic to the plasma membrane, maintained normal expression levels and turnover relative to WT *DSG1*, but behaved as a dominant-negative mutant, possibly owing to compromised raft association. Extradesmosomal DSG1 might undergo novel protein interactions (blue circles and ovals). Ultimately, this may lead to a defective barrier, altered differentiation, and compromised cell mechanics. This figure was created with BioRender.com. DSG, desmoglein; ER, endoplasmic reticulum; ERK, extracellular signal–regulated kinase; GJ, gap junction; MEK, MAPK/extracellular signal–regulated kinase kinase; PPK, palmoplantar keratoderma; SAM, severe dermatitis, multiple allergies, metabolic wasting; TJ, tight junction; WT, wild-type.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

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