Lipid Rafts and Bullous Diseases

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Abstract: Multiple observations point to involvement of lipid membrane domains, known as lipid rafts, in the pathology of human disorders. The putative role of lipid rafts in hereditary and acquired skin blistering diseases is discussed in this review. Stable adhesion of the epidermis to the underlying basement membrane is secured by hemidesmosomes, specialized multiprotein complexes in basal keratinocytes. Loss of function of hemidesmosomal proteins due to inherited or acquired abnormalities result in weak dermal-epidermal adhesion and blistering of the skin. Lipid rafts regulate biological functions of two hemidesmosomal transmembrane components: collagen XVII and α6β4 integrin. Ectodomain shedding of collagen XVII is regulated by membrane lipid domains, suggesting involvement of lipid rafts in the pathogenesis of junctional epidermolysis bullosa, a genetic disease caused by mutations in the collagen XVII gene, and of bullous pemphigoid, an autoimmune disease with autoantibodies to this collagen. Similarly, adhesive and signaling functions of α6β4 integrin are modulated by lipid rafts, again linking lipid rafts to junctional epidermolysis bullosa. Therefore, modulation of lipid domains in the epidermis might have therapeutic potential for this group of skin blistering diseases.

Keywords: Bullous pemphigoid, collagen XVII, epidermolysis bullosa, hemidesmosome, lipid raft, α6β4 integrin, skin blistering.

INTRODUCTION

Increasing evidence points to the role of lipid rafts in a variety of pathologic conditions [1] including Alzheimer's [2] and Parkinson's [3] disease, cardiovascular [4, 5], autoimmune [6, 7], infectious [8], inherited [9] and prion disorders. These findings make lipid domains an attractive target for pharmacological trials for treatment and prevention of different diseases. Recent investigations suggest involvement of lipid raft signaling also in the pathogenesis of inflammatory [10] and inherited skin diseases [11, 12], although not very much is known about such events yet. This chapter focuses on what could be the role of lipid rafts in the molecular pathology of skin blistering.

The organization of lipids in the biological membranes can be best explained as mosaic of lipid domains of variable size, so-called lipid rafts [13], highly dynamic cholesterol and sphingolipid enriched domains of plasma membrane, with the size of 10-200 nm. The formation of the isolated lipid domains within the membrane occurs due to different packing capability of sphingolipids and glycosphingolipids (GPL) in the membrane. Sphingolipids contain long saturated acyl chains that allow them to pack together more tightly than unsaturated GPL and thus separate to isolated domains. Cholesterol binds preferentially to sphingolipids and favours the lipid phase separation [14, 15]. Because of their different density and melting point lipid rafts can float in the more-liquid GPL-rich bulk of the plasma membrane and cholesterol can shuttle between the raft and non-raft phase, having the higher affinity to rafts [16]. For more details on the structure and functions of lipid rafts in skin cells, the reader is referred to Chapter 1.

Importantly, lipid rafts can include or exclude membrane proteins to variable extents. Proteins with raft affinity include GPI-anchored proteins, doubly acylated proteins, cholesterol-linked and palmitoylated proteins, and many transmembrane proteins [17]. The mechanisms of molecular targeting of transmembrane proteins to lipid raft domains is not yet fully understood, but mutational analysis has shown that amino acids in the transmembrane domain near the exoplasmatic leaflet of the membrane are critical for this process [18]. The principle by which rafts exert their functions is a compartmentalisation of specific membrane proteins in the lipid domains. Rafts are dynamic, and both proteins and lipids can move in and out of raft domains with different speed [17]. This variable distribution of proteins between lipid subcompartments could regulate protein-protein interactions within plasma membrane and affect their functions. Small rafts can contain only a few proteins [17], so to engage in membrane function, they have to cluster together to form the large platform, where the participants in signal transduction process can meet [19, 20].

Lipid Rafts Regulate Keratinocyte Functions: Lipid rafts are involved in a broad spectrum of cellular events in epidermal biology. In human keratinocytes, lipid domains were first detected using imaging techniques by labelling with cholera toxin B subunit [21, 22], and they have since been shown to play a role in TNFα– [10], EGFR- [23] and cell adhesion associated signaling [11, 12, 24, 25]. They also regulate intercellular communication *via* gap junctions [26] and keratinocyte differentiation [27, 28]. Already these few

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roles indicate the importance of lipid rafts in maintenance of tissue integrity and morphology in the skin, cell growth and survival, modulation of cell behaviour, as well as in inflammatory processes and wound healing. Cholesterol, the major component of lipid rafts, is an important constituent of the cornified layer, as it contributes to epidermal lipid barrier in the skin [29, 30]. Epidermal differentiation involves a spatially and temporally tightly regulated expression of differentiation-related proteins, and modulation of membrane cholesterol has been shown to alter the expression of epidermal differentiation markers keratin 14 and keratin 10, and to result in the phenotype of early differentiating keratinocytes [28].

Hemidesmosomes Mediate Keratinocyte Adhesion: Adhesion of basal keratinocytes to the underlying basement membrane is secured by hemidesmosomes [31, 32], specialised multiprotein complexes that link keratin cytoskeletal elements through transmembrane components to the extracellular matrix proteins. Hemidesmosome- mediated stable attachment of basal keratinocytes plays a crucial role in maintaining skin integrity. This is indirectly demonstrated by the fact that loss of hemidesmosome function leads to skin blistering in inherited or acquired human diseases and in mouse models.

Two transmembrane proteins, α6β4 integrin and collagen XVII, play a crucial role in the formation and maintenance of hemidesmosomes. Interaction of α6β4 integrin with plectin is one of the first steps in the hemidesmosome formation [33] and important for their mechanical stability. α6β4 integrin appears to be essential for cell adhesion, it connects keratinocytes to one of the major components of the basement membrane, laminin 332 [34]. Collagen XVII belongs to the group of transmembrane collagenous molecules [35] and contributes into maintenance of epidermal-dermal integrity. The intracellular domain of collagen XVII is necessary for the stable attachment of hemidesmosomes to keratin intermediate filaments [36, 37], and the extracellular domain of collagen XVII can bind to α6 integrin and laminin 332, and it guides correct integration of laminin 332 into the pericellular matrix [38].

Lipid Rafts and Hemidesmosomal Transmembrane Proteins: α6β4 integrin localizes to lipid rafts as a result of palmitoylation of the β4 subunit, and compartmentalization in lipid rafts potentially explains several aspects of α6β4 signaling [12]. Palmitoylation-deficient α6β4 integrin did not associate with rafts or activate SFK (Src family kinase) signaling and failed to promote keratinocyte proliferation in response to EGF, implying that mitogenic signaling requires α6β4 integrin incorporation into lipid rafts. Furthermore, α6β4 integrin preferentially binds pSFK in lipid rafts. Palmitoylation is a reversible process, allowing for regulated incorporation of α6β4 into lipid rafts. Binding to its physiological ligand, laminin 332, or antibody induced oligomerization of α6β4 increased the amount of integrin recovered in the raft fraction, suggesting that extracellular matrix (ECM) binding of α6β4 integrin increases its affinity for lipid rafts and enhances signaling. Several other integrins (α5β1 α6β1, α6β4) have also been shown to bind lipid rafts, and functional activation of integrins appears to be linked to the lipid raft localisation [39-41]. One interesting investigation showed that the membrane order itself, i.e. the fluidity of plasma membrane, is highly sensitive to cell-ECM adhesion and to integrin clustering [42]. These data indicate that a significant portion of raft structure is protein dependent, and it is likely that both lipids and proteins cooperate to establish and maintain ordered membrane domains at focal adhesions and to stimulate downstream signaling.

Collagen XVII exists in two forms, as a full length protein and as a soluble ectodomain [43]. Its collagenous ectodomain can be proteolytically released from the cell surface. The cleavage occurs close to the plasma membrane, and is mediated by metalloproteases of the ADAM (A Disintegrin And Metalloprotease). ADAM-17/TACE (TNF-a Converting Enzyme) appears to be a major sheddase of collagen XVII [44]. Collagen XVII localises into lipid domains, and membrane sublocalization of collagen XVII plays a key role in the regulation of its shedding [11]. Distribution of plasma membrane cholesterol strongly affects shedding of collagen XVII, since alteration of cholesterol content increased the proteolytic release of the collagenous ectodomain from the cell surface. TACE is non-raft protease, whereas the majority of collagen XVII molecules are localised in lipid rafts and thus inaccessible for enzymatic processing by TACE (Fig. **1)**. After disorganisation of the lipid domains collagen XVII molecules become available for TACE, which results in enhanced shedding. Therefore, incorporation of collagen XVII into lipid domains seems to provide an important mechanism to modify accessibility to the sheddases and could regulate interactions of the cell with extracellular ligands. Interestingly, low cholesterol has been shown to promote early keratinocyte differentiation. It seems feasible that this could occur through lipid raft-mediated regulation of collagen XVII shedding, which releases the keratinocytes from their binding partners within the underlying basement membrane. Lipid domains can also regulate ectodomain shedding of other transmembrane collagens, including types XIII [45] and XXIII [46], processed by furin. It seems that lipid domains provide the general mechanism to modulate proteolytic processing of transmembrane collagens.

Genetic Defects of Epidermal Adhesion. Mutations in the genes encoding α6β4 integrin (*ITGA6 and ITGB4*) and collagen XVII (*COL17A1)* are associated with junctional epidermolysis bullosa (EB), an group of genetic skin blistering disorders with variable clinical phenotypes [47-49]. Functional consequences of the gene defects include diminished epidermal adhesion and skin blistering in response to minimal trauma or shearing forces. Morphological characteristics of junctional EB are rudimentary hemidesmosomes and subepidermal tissue separation. Clinical hallmarks, in addition to blisters and erosions of the skin and mucous membranes, include nail dystrophy, loss of hair, and dental anomalies (Fig. **2**).

Thus far, more than 50 different *COL17A1* mutations have been disclosed in patients with junctional EB. Studies on genotype-phenotype correlations have not only revealed the molecular pathomechanisms in this genodermatosis but have augmented our understanding of the normal functions of collagen XVII and its individual subdomains [35, 37, 38, 50, 51].

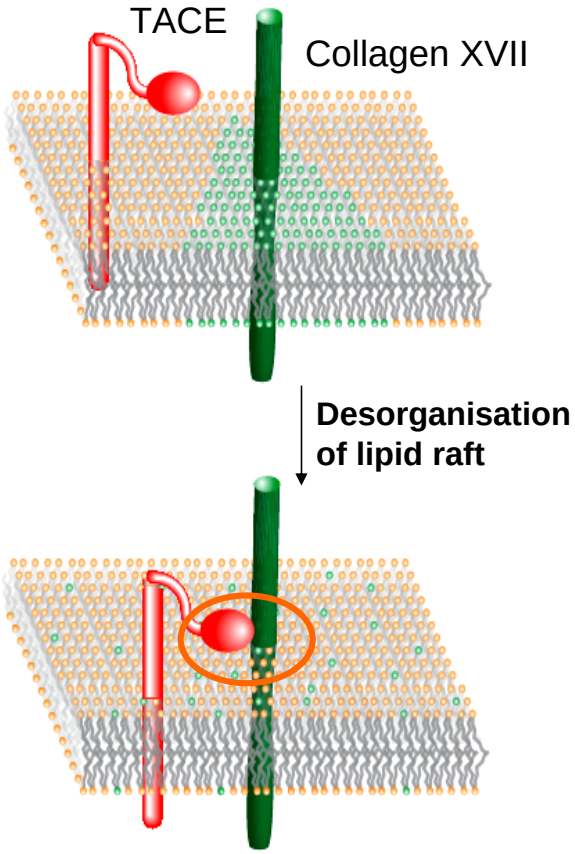

Fig. (1). Integration into lipid rafts regulates shedding of collagen XVII. Collagen XVII mainly associates to lipid rafts, this association provides a key for regulation of its shedding by controlled accessibility to TACE. TACE is not a raft-associated protein, and collagen XVII molecules, mostly in the rafts, are inaccessible for TACE. After disruption of the lipid rafts, collagen XVII molecules become available for enzymatic processing by TACE and shedding is increased.

Ablation or disruption of the genes for the integrin α6 or β4 subunits lead to variably severe cases of junctional EB with pyloric atresia. Most mutations target the gene encoding the integrin β4 subunit, fewer mutations have been found in the gene for the α6 subunit. A consequence at the ultrastructural level is that hemidesmosomes are absent or hypoplastic. Nonsense mutations result in the absence of the integrin and abolition of all its functions, including binding to laminin 332 and recruitment of plectin to the hemidesmosomal plaque. Missense mutations resulting in amino acid substitution in the plectin-binding region of the integrin β4 intracellular domain have been reported to cause milder phenotypes [52].

Significant phenotypic variation of clinical phenotypes in junctional EB still remains poorly understood and has been attributed to genetic modifiers, environmental factors, random events, and interactions between any of these sources [53, 54], but all these are presumptions. In this context there may well be the role of membrane lipids and lipid rafts on the clinical phenotype of EB since both collagen XVII and α6β4 are lipid raft proteins. As described below, the ligand interactions of collagen XVII and α6β4 with other proteins or enzymes in the cell membrane may contribute to the pathogenesis of the skin blistering diseases.

Lipid rafts modify epitopes in autoimmune bullous skin diseases. Pemphigoid diseases are a group of acquired autoimmune blistering skin diseases. In bullous pemphigoid (BP), the most frequent autoimmune blistering disorder of the skin, autoantibodies target collagen XVII [55]. The immunodominant epitopes are located within the juxtamembranous NC16a-subdomain [56, 57]. As shown by passive transfer mouse models and *in vitro* models, antibodies against collagen XVII are pathogenetically relevant, since their binding to the antigen induces complement activation leading to neutrophil infiltration, secretion of proteases and to epidermal-dermal separation [58].

In a subset of BP, the linear IgA dermatosis (LAD), the ectodomain of collagen XVII is specifically targeted by IgA-autoantibodies. LAD is characterized by pruritic tense blisters and by tissue-bound and circulating IgA-autoantibodies against the epidermal basement membrane zone. In addition to the shed 120 kDa ectodomain of collagen XVII, the IgA-autoantibodies recognize a 97 kDa antigen, which represents a truncated form of the collagen XVII ectodomain.

Lipid rafts have previously been shown to be involved in the pathogenesis of other autoimmune diseases. In systemic lupus erythematosus (SLE), a multiorgan autoimmune

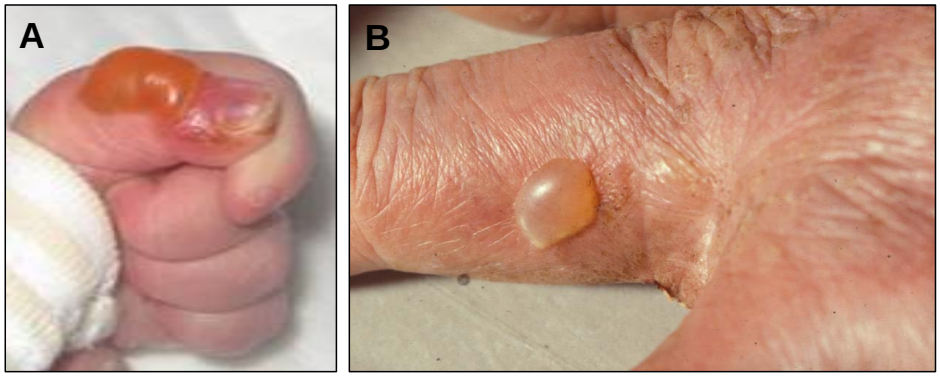

Fig. (2). Skin blistering as a consequence of loss of collagen XVII function in junctional epidermolysis bullosa in an infant (**A**) and in bullous pemphigoid in an adult (**B**).

disease, they can affect T cells signalling [6, 7]. Lymphocyte-specific protein tyrosine kinase, critical for maintaining the resting state of T cells, is mislocalised in SLE T lymphocytes [6]. In normal T cells, the lymphocyte-specific protein tyrosine kinase is mainly localised outside the lipid rafts, but in SLE T cells it associates with the rafts. Another study proved the role of lipid rafts in modulation of higher calcium responses in SLE T cells [7]. A link has been suggested between lipid raft dependent ectodomain shedding of CD30 and certain autoimmune diseases, such as systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, atopic dermatitis, Wegener's granulomatosis, Graves' disease, and Hashimoto's thyroiditis, which are associated with increased serum levels of soluble CD30 ectodomain [59].

In both BP forms, the immunodominant epitopes are localized within the NC-16a subdomain, which harbours the sheddase cleavage site. Both this domain and its proteolytic cleavage seem to play an important pathogenetic role, since shedding of collagen XVII generates neoepitopes, as demonstrated by the fact that the new N-terminus of the shed ectodomain presents a particularly strong epitope for IgG autoantibodies in BP and IgA autoantibodies in LAD [60, 61].

Thus, all events regulating shedding of collagen XVII, such as lipid rafts or phosphorylation of the cleavage site [11, 62], can also influence the pathology in autoimmune blistering skin diseases. It is intriguing to hypothesize that inhibitors of shedding or agents modulating lipid rafts organisation may have therapeutic potential for this group of skin diseases.

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ABBREVIATIONS

ADAM = A Disintegrin And Metalloprotease

BP = Bullous Pemphigoid

EB = Epidermolysis Bullosa

EGFR = Epidermal Growth Factor Receptor

GPL = Glycosphingolipids

LAD = Linear IgA Dermatosis

SFK = Src Family Kinase

SLE = Systemic Lupus Erythematosus

TACE = TNF-α Converting Enzyme

TNFα = Tumor Necrosis Factor Alpha

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