Commentary & View

Is DRM lipid composition relevant in cell-extracellular matrix adhesion structures?

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Focal adhesions mediate cell-extracellular matrix adhesion. They are inserted in detergent-resistant membrane microdomains enriched in phosphatidylinositol-4,5-bisphosphate. In spite of the relevance that membrane lipids appear to have on cell adhesion structures, to our knowledge, there are no previous reports on the membrane lipid composition where focal adhesions are located in vivo or on how changes in local membrane composition contribute to focal adhesion maintenance. This may be due to the fact that the explosion of information in the fields of genomics and proteomics has not been matched by a corresponding advancement of knowledge in the field of lipids. The physiological importance of lipids is illustrated by the numerous diseases to which lipid abnormalities contribute. To gain insight into the role of membrane lipid composition in the preservation of epithelial cell adhesion to the substratum, how specific changes in the membrane lipid composition in vivo affect the maintenance of focal adhesions in renal papillae collecting duct cells has been previously studied. It is currently considered that phosphatidylinositol-4,5-bisphosphate plays a crucial role in the maintenance of assembled focal adhesion. However, such pool of polyphosphoinositides is yet to be part of a domain of a specific lipid composition to serve as a membrane lipid stabilizing the focal adhesion plaque.

The correct functioning of an organ depends on its tissue organization which, in turn, depends on the structures that enable cells to attach to the extracellular matrix (ECM). The best characterized cell-extracellular matrix (ECM) adhesions are the focal adhesions (FAs). FA assembly occurs by the binding of the integrin-extracellular domain to the ECM proteins followed by the interaction of the β-integrin cytoplasmic domain with talin, which, in turn, binds vinculin.1 Vinculin can bind to more than ten different partners so, the theoretical number of different combinations of molecular interactions involved in linking integrin to actin is enormous.1 Talin and vinculin need to be inserted into the membrane phospholipid bilayer to form FA plaques. Talin binds to membrane phospholipids by exhibiting both a hydrophobic and an electrostatic component.2-4 Vinculin is one of the few proteins for which binding to lipids has been demonstrated in vitro and in vivo.5,6 Vinculin is auto-inhibited by an intramolecular interaction between the head and tail regions that blocks the association of the free (and not the membrane-associated) form of vinculin with most of its protein ligands.7,8 The recently solved crystal structure of the vinculin tail suggests that the activation of vinculin-ligand binding requires the simultaneous binding of several ligands.9 In vitro, the interaction between the N- and C-terminal regions of vinculin can be relieved by charging the vinculin tail domain with phosphatidylinositol-4,5-bisphosphate [PI(4,5)P2].10,11 Binding of PI(4,5)P2 to vinculin can stabilize vinculin’s localization to adhesion sites and facilitate its interaction with other functional components.12

On the other hand, it has been reported that many of the molecular components that regulate cell-ECM adhesion are associated with cholesterol and sphingolipid-enriched detergent-resistant membrane microdomains (DRM), which are also enriched in the acidic phospholipid PI(4,5)P2.13,14 In spite of the relevance that membrane lipids appear to have for FA assembly, to our knowledge, there are no previous reports on the membrane lipid composition where FAs are located in vivo or on how changes in local membrane composition contribute to FA maintenance.

To gain insight into the role that membrane lipid composition plays in the preservation of epithelial cell adhesion to the ECM, how specific changes in the membrane lipid composition in vivo affect the maintenance of FAs has been previously studied. To this end, the investigations were carried out with primary cultured rat renal papillae collecting duct cells that were not genetically manipulated. FAs have been previously associated with DRM and also with local membrane accumulation of PI(4,5)P2, and it has been recently shown that they can regulate membrane order.15-16 However, a direct demonstration of the presence of FAs in such resistant domains and their biochemical characterization has not been reported before. By using a two-step centrifugation procedure and changing sucrose concentration, Triton X-100 resistant membrane microdomains from the microsomal fraction of primary cultured collecting duct cells were isolated, where FA complexes are located.17 In such DRM, it has been found that vinculin and talin are bound to PI(4,5)P2 and that both proteins interact, as assessed by immunoprecipitation analysis.
The presence of vinculin bound to PI(4,5)P₂, together with talin-vinculin association in DRM, constitutes the biochemical expression of the existence of FA complexes in DRM isolated from rat renal papillae. Once established that FA complexes were located in DRM, the DRM lipid composition was characterized in comparison with the Triton X-100 soluble fractions. Cholesterol concentration was ten times higher in DRM than in the Triton X-100 soluble fraction. As regards the phospholipid profile, DRM showed a 70% increase in sphingomyelin, accompanied by a 30% increase in phosphatidylethanolamine and a 40% decrease in phosphatidylserine, as well as no differences in phosphatidylcholine and phosphatidylglycerol.

The study of individual phospholipid fatty acid composition revealed that DRM are enriched in arachidonic acid mainly due to phosphatidylethanolamine, which doubled its content with respect to phosphatidylethanolamine in the Triton X-100 soluble fraction. The quantity of DRM-polyphosphoinositides, PI(4)P and PI(4,5)P₂, was several times higher than in the soluble fraction, denoting the massive accumulation of these acidic phospholipids in renal papillary collecting duct cell DRM. It is known that PI(4)P and PI(4,5)P₂ are formed by phosphatidylinositol phosphorylation by specific kinases. Polyphosphoinositide synthesis in DRM was twice higher than in the Triton X-100 soluble fraction, as assessed by [32P]-Pi incorporation into phospholipids, denoting that DRM-associated phospholipids were metabolically active. The high polyunsaturated phosphatidylethanolamine content, together with the enrichment in polyphosphoinositides, suggests that such DRM correspond to the inner leaflet of the plasma membrane, which is consistent with the fact that FAs are the cytoplasmic face of the cell contact site. It has been proposed that the lipid composition of such DRM corresponds to the physiological environment where FAs are inserted.

Previous in vitro studies have demonstrated that the insertion of either vinculin or talin into the lipid bilayer depends on the bilayer phospholipid composition. In order to study the influence of membrane lipid composition on FA preservation in vivo, membrane-affecting agents, such as methyl-β-cyclodextrin (CDex), neomycin and LiCl were used. FAs cannot be detected in histological preparations from intact tissue, but can be observed in cultured cells. Therefore, we took advantage of the fact that cultured papillary collecting duct cells preserve their tendency to interact with their self-formed ECM by mimicking their behavior in intact tissue. We thus established a parallelism between the biochemical data obtained from papillary microsomes and the morphological observations from immunofluorescence confocal microscopy performed in primary cultures of collecting duct cells. By using such experimental strategy, it has been demonstrated that membrane lipid composition affects the in vivo preservation of FAs, as clearly shown by the immunofluorescence images, where vinculin-stained FAs were dissipated (Fig. 1). The various membrane-affecting agents differently affected FAs, depending on their capacity to change the membrane lipid composition. Consistent with previous reports in smooth muscle cells, the treatment with CDex did not lead to DRM elimination. Instead, CDex induced a decrease in cholesterol but also a membrane lipid redistribution. In fact, a net increase in the sphingomyelin and phosphatidylserine content in DRM, counterbalanced by a decrease in phosphatidylcholine and phosphatidylethanolamine content, was observed. In contrast, PI(4)P and PI(4,5)P₂ did not change significantly. It has been reported that DRM serves as a platform for PI(4,5)P₂, and that such PI(4,5)P₂ accumulation serves to stabilize the assembled FAs after binding to vinculin. However, although polyphosphoinositides were still accumulated in DRM after CDex treatment, FA proteins such as vinculin and talin, did not persist assembled to form FAs (Fig. 1).

Figure 1. Immunofluorescence images showing the effect of changes in DRM lipid composition on vinculin-stained focal adhesions. (A) Cultured collecting duct cells labeled for vinculin, displaying typical focal adhesions. (B–D) Cultured collecting duct cells treated with 5 mM methyl-β-cyclodextrin, 1 mM neomycin or 10 mM LiCl, respectively. Note the dissipation of vinculin-stained FAs [compare with non-treated cells in (A)]. Arrowhead: focal adhesions. Bars: 20 μm.
it is known that LiCl blocks phosphoinositide synthesis. Apart from the expected decrease in PI(4)P and PI(4,5)P₂ DRM content, LiCl evoked an increase in cholesterol and sphingomyelin content, accompanied by an important phosphatidylinositol enrichment. It is important to note that the treatment of cultured collecting duct cells with CDex and neomycin induced an important decrease in the number of vinculin—as well as talin—stained FAs, whereas LiCl only evoked a decrease in vinculin-stained FAs. It is known that FAs are hierarchical structures where additional FA proteins assemble as they mature. Talin is the first protein that forms FAs, whereas vinculin is added thereafter. Thus, even without vinculin, talin-containing FAs can still be present, thus conforming the minimal FA assembling. It is known that vinculin has to be bound to PI(4,5)P₂ to stay in FA plaques. The selective dissipation of vinculin from assembled FAs can be due to their different affinities for membrane lipids. Thus, vinculin binding to PI(4,5)P₂ is of low affinity, whereas binding to phosphatidylinositol is of high affinity but does not serve to assemble vinculin into FAs. Thus, the lowering in PI(4,5)P₂ content, together with the high increase in phosphatidylinositol produced by LiCl, may account for the dissipation of vinculin from FAs. By contrast, talin has high affinity to bind PI(4)P and thus the decreased concentration of DRM—PI(4)P could still be enough to bind talin. On the other hand, it appears that the new DRM conformation, where cholesterol and sphingomyelin increased their concentration, could result favorable to stabilize talin-containing FAs. It is currently considered that PI(4,5)P₂ is the membrane phospholipid that plays the main role in the maintenance of assembled FAs. However, it has been observed that such pool of polyphosphoinositides has to be part of a domain of specific lipid composition to serve as a membrane lipid that stabilizes FA plaques. Among membrane lipids and taking into consideration that the decrease in cholesterol was a common feature in the deleterious effect of CDex and neomycin, we suggest that cholesterol is in fact the crucial lipid for evolving the lipid environment to maintain the FA plaques assembled. In accordance with our findings, a recent report has concluded that membrane order at FAs depends on cholesterol.

We used the various membrane-affecting agents only as a tool to study the influence of membrane lipid composition on FA maintenance. However, it is interesting to point out that both neomycin and LiCl are pharmacological agents of known nephrotoxic effects. In this context, our observations could also be pharmacologically relevant. Indeed, neomycin is an aminoglycoside antibiotic known to cause tubular necrosis. Thus, the disruption of the cell-ECM adhesion described above could be an explanation for the deleterious effect caused by neomycin treatment since it is known that epithelial cells have to be bound to the extracellular matrix in order to survive and not die by anoikis. In addition, alterations in PI(4,5)P₂ have been suggested to perturb membrane lipid asymmetry in Scott syndrome. On the other hand, LiCl is used for the treatment of some human mental diseases and it is known that long-term treatment with this agent provokes alterations in the renal capacity for concentrating urine. Although the pharmacological dose of LiCl is lower (0.8–1 mM) than the concentration used in our experiments, chronic low doses may also affect FAs. The impairment of vinculin-containing FAs described above could be a primary cause that affects cell-ECM adhesion progressively, thus affecting renal function. Moreover, the alteration in membrane lipid composition, mostly cholesterol enrichment, could disturb the location or the function of the membrane transporter systems involved in normal renal tubular physiology, thus affecting the urine concentration process.

In conclusion, our work contributes with new evidences to the importance of the lipid composition of the specific membrane lipid domain, where FAs are included, and suggests that it may be relevant for the maintenance of the structures that tether the collecting duct epithelium to the extracellular matrix.

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