The effect of MPP1 on organization of resting state rafts in plasma membrane

SUMMARY

Plasma membrane can no longer be perceived as it was proposed by Singer & Nicolson, as a homogenous mosaic of fluid lipid and integral membrane proteins. Since then, extensive research have shown that plasma membrane contains dynamic structures, called microdomains/rafts existing in different time and space scales, controlled by various protein-protein, lipid-protein and primarily lipid-lipid (mainly cholesterol and sphingomielin) interactions. These domains are continuously formed and dispersed within the plasma membrane enabling time and space compartmentalization of what and functional diversity. Lateral membrane structure has been extensively investigated for the last fifteen years since lipid/membrane-raft hypothesis has been formulated. Currently membrane rafts can be described as dynamic, short-living, sterol-enriched, ordered nanoscale assemblies of proteins and lipids which could be cross-linked and stabilized to form larger, more stable complexes (resting-state rafts) to facilitate their function. Despite the fact that every year lateral membrane heterogeneity seems increasingly more precisely characterized, still not a lot is known about molecular mechanism underlying resting state rafts formation/disorganization in native membranes.

In the previous work, using standard methods in raft research, we showed, that MPP1 (membrane palmitoylated protein 1/p55), a MAGUK-family protein, affects lateral membrane organization of erythrocytes and erythrocyte precursors, proposing one of the first mechanism where single protein not only partitions into the membrane rafts but actually triggers their assembly. So far the major, characterized role of MPP1 in erythrocytes was its ability to link erythrocyte membrane skeleton to the membrane bilayer by formation of a tripartite complex with protein 4.1 and glycophorin C. We were able to show that it actually plays a crucial role in erythroid lateral membrane organization. Here we decided to test the above-mentioned hypothesis and further explore the role of MPP1 in the resting state raft formation using more precise, modern methods enabling direct membrane structure observation.

Due to their small size (~20 nm) and highly dynamic nature, membrane microdomains are not easy to visualize or purify making the studies on raft structure and function challenging. Recently, methods for microscopic observation of phase separation in plasma

membrane vesicles have been established. Giant plasma membrane vesicles (GPMVs) reflect live cell membrane deprived of cytoskeleton and confirms ability of naturally complex membranes to phase separate enabling direct observation of membrane lateral organization. They provide unique tool for investigation of membrane structure and order. GPMVs were used to analyze the effect of MPP1 on membrane fluidity and phase separation.

Results of this study show direct effect of a single protein on the membrane properties. The reduction of MPP1 content leads to changes in phase separation abilities of plasma membrane vesicles, revealed as marked decrease in miscibility transition temperature, what clearly points to a role of MPP1 in coalescence of naturally occurring nanoassemblies in the native membrane into larger resting state rafts. Additionally, C-laurdan general polarization and di-4 lifetime value measurements confirmed significant changes in membrane fluidity and less ordered state of vesicles deprived of MPP1. As the observed changes in membrane order could be a result of changes in GPMVs lipids composition and not the deficiency of MPP1 we decided to isolate GPMVs and perform analysis of their lipid composition. Both, analysis of major lipid classes content in GPMVs isolated from all three cell types as well as GP measurements of liposomes prepared from lipids extracted from obtained vesicles did not reveal any noticeable differences strengthening our hypothesis that binding of MPP1 to the membrane affects membrane fluidity. Additionally, to validate whether the observed changes in membrane order can be reversed, and are not a result of the off-target effect of *MPP1* gene silencing, we performed a rescue in knockdown cells. MPP1 synthesis was successfully restored in KnD cells, resulting in GPMVs membrane order recovery observed as increased di-4 lifetime values comparable to those obtained for control-derived GPMVs. Presented here studies document a novel, remarkable role of MPP1, as a scaffold molecule responsible for plasma membrane lateral organization of erythroid cell and supports one of the first described biological mechanisms of membrane resting state rafts formation.

Our data suggests that membrane of constant lipid composition can modulate domain physicochemical properties by protein like MPP1 and therefore regulate the functional role of the resting state domains. To prove, that observed membrane fluidity changes have functional implications we decided to test the activation of well characterized “raft-dependent” membrane receptors. We were able to show that in cells in which the expression of *MPP1* have been silenced signal transduction from two RTK receptors, insulin receptor and c-kit (stem cell factor receptor) was impaired. Interestingly receptors activation, observed as their autophosphorylation, occurred to the same level in all analysed cell types, however, further signal transduction was inhibited, shown as reduced level of efector kinases phosphorylation

(Erk1/2). Further analysis showed, that in MPP1 KnD cells the activation of small GTPase, Ras was diminished, which strongly correlates with association of this adaptor protein with activated receptors in membrane rafts. In MPP1 deficient cells we did not observe nucleotide GDP to GTP exchange in Ras protein, what resulted in impaired further signal transduction. This is the first proof of H-Ras dependence on MPP1.

Obtained results document that MPP1 is crucial for proper lateral membrane organization and point to physiological importance of observed mechanism for erythroid cell membrane function. They suggest that MPP1 influence nanocomplexes agregation/oligomerization and resting state raft stabilization.

Presented study assured us that all observed changes in lateral structure of plasma membrane in erythroid cells are dependent on single protein, MPP1, which is a novel function of this MAGUK family protein, but also one of the first cases describing single protein involvement in lateral plasma membrane organization. Our hypothesis, which is now a subject of further research, is that MPP1 binds to preexisting cholesterol/protein nanocomplexes/clusters, induces their clustering and stabilization into resting state rafts/microdomains making them functional. As impaired formation of membrane microdomains could underlay serious disorder, i.e. unique hemolytic anemia and influence important cell processes such as signaling and membrane trafficking which are broadly upregulated for instance in cancer. Unraveling of mechanism of rafts formation opens new possibilities for modulation of cell functioning and targeted therapies. Moreover, discovering of a new function of MPP1, a MAGUK family protein, creates new prospects for further research considering MPP1 homologues, other MAGUK proteins, as potential candidates responsible for maintaining proper membrane structure.