Flippases and scramblases regulate phospholipid distribution in the plasma membrane during platelet activation and apoptosis

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Asymmetric distribution of phospholipids in the plasma membrane is sustained by flippases (belonging to the P4 ATPase family) in healthy cells and disrupted by scramblases during certain physical or pathological processes. In platelets, surface exposed phosphatidylserine (PS) not only acts as an ‘eat-me’ signal recognized by phagocytes during apoptosis, but can also act as a physiological signal initiating blood coagulation when platelets are activated. However, the mechanism whereby flippases and scramblases regulate phospholipid redistribution during platelet activation and apoptosis is still unclear.

We first identified ATP8A1 and ATP11B as the two predominant flippases in mouse platelets. Furthermore, they are inactivated by caspase and calpain action during apoptosis and activation, respectively. This is thought to contribute to PS exposure in these two biological processes. However, flippase inactivation alone is not sufficient for phospholipid redistribution, but requires scramblase activation, as well. We utilized a fluorescent NBD (7-nitro-2-1,3-benzoxadiazol-4-yl) labeled phospholipid uptake assay to measure scramblase activity and found that scramblases were activated by two distinct pathways during platelet activation and apoptosis. The former is calcium dependent but caspase independent, while the latter is calcium independent and caspase dependent.

It has been shown that TMEM16F is the main calcium-dependent scramblase in platelet activation, but the molecular identity of the caspase dependent scramblase in apoptosis remains unknown. We could exclude the scramblase candidate Xkr8 due to lack of expression in platelets as ascertained by RT-PCR. We subsequently tested the presence and activation of known scramblase candidates in platelets.

Taken together, we found a complex array of flippases and scramblases that regulate phospholipid distribution in the plasma membrane during platelet activation and apoptosis. This may shed light on the mechanism of platelet associated cell death and blood coagulation disorders.