Role of platelet autophagy in cardiovascular diseases

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ABSTRACT

Platelet hyperactivity leads to thrombosis, the primary cause of cardiovascular and cerebrovascular adverse events. Emerging evidence suggests that autophagy plays a fundamental role in platelet function, both at baseline and in response to stress. Autophagy is an intracellular mechanism devoted to the removal and recycling of damaged cytoplasmic cargoes. Basal level of autophagy ensures physiological platelet aggregation and hemostasis. Autophagy is impaired in platelets isolated from subjects at high cardiovascular risk whereas autophagy stimulation reduces platelet hyperactivity, along with an overall amelioration of oxidative stress status. In this mini-review, we explore the current literature on the role of autophagy in platelet biology and its relevance as a therapeutic target for counteracting cardiovascular diseases.

Review

Platelets play a fundamental role in physiological hemostasis and pathological thrombosis.[1] They are also involved in the host response to injury, inflammation, infectious diseases, and hematological malignancies,[2] by sensing external signals and developing an appropriate response through the activation of several molecular mechanisms. Platelet hyperactivity is the hallmark of thrombosis, which is a leading cause of heart attack and stroke.[3] During thrombosis, platelets adhere to immune cells and exposed subendothelial matrix and plaque components, activating and releasing pro-thrombotic substances. The latter contributes to platelet aggregation and the formation of a thrombus. Platelets release mediators that activate inflammatory pathways, oxidative stress, and apoptosis, ultimately leading to endothelial dysfunction.[4,5] However, despite enormous efforts to understand the mechanistic basis of platelet function and thrombosis, major challenges remain to improve therapy. Emerging evidence demonstrated that autophagy has a great relevance in platelet function.[1] Autophagy is an evolutionarily-conserved intracellular process by which dysfunctional or senescent cytoplasmic elements, including whole organelles, are sequestered in double-membrane vesicles termed autophagosomes that then fuse with lysosomes to form the autophagolysosomes, in which the cytoplasmic cargo is degraded. Essential elements derived from lysosomal digestion are recycled and reintroduced in cell metabolism.[6] Recent evidence highlighted the relevance of autophagy defects in human disease.[7] Autophagy ensures cardiovascular homeostasis at baseline and limits cardiac and vascular injury in response to stress, as observed in murine models of myocardial infarction, metabolic cardiomyopathy, heart failure, and stroke by acting on cardiomyocytes and endothelial cells.[8,9] Autophagic markers were also observed in platelets through the detection by immunological assays of microtubule-associated protein 1A/1B-light chain 3 (LC3) and sequestosome 1 (SQSTM1).[1] As in nucleate cells, nutrient deprivation induces autophagy in platelets.[1] The impairment of autophagic flux was reported in platelets isolated from patients with sepsis or with Vici syndrome, a rare hereditary disease characterized by the deficiency of Ectopic P granules protein 5 homolog (EPG5), a protein involved in the fusion between lysosomes and autophagosomes.[10]

Platelet autophagy inhibitors, both pharmacological and genetic, impair platelet aggregation and adhesion.[1] Other studies demonstrated that inhibition of autophagy in platelets leads to impaired hemostasis and thrombus formation in the FeCl3-induced carotid injury mouse model.[11] These results suggest that the disruption of autophagy impairs platelet function.[1,11]

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Autophagy induction by starvation above basal level reduces platelet aggregation *ex vivo*.[12] We recently found that patients at high cardiovascular risk, such as smokers, patients with metabolic syndrome or those with atrial fibrillation show reduced levels of endogenous platelet autophagy, compared to healthy subjects.[13] Autophagy impairment in these conditions is associated with increased platelet aggregation, a strong contributor to cardiovascular and cerebrovascular events that occur in smokers, in patients with metabolic syndrome or atrial fibrillation. Notably, reactivation *ex vivo* of autophagy through a mixture of natural activators of autophagy can reduce platelet aggregation (Figure 1). Natural compounds used to activate autophagy in this study include the non-reducing disaccharide trehalose, polyamine spermidine, nicotinamide, and polyphenols. The latter act in a synergistic manner by modulating different regulators of autophagy, such as transcription factor EB (TFEB), histone acetyltransferase EP300 and NAD+ levels. For this reason, the pro-autophagic and anti-aggregating effects of this mixture were more pronounced compared to a single compound of the mixture tested alone.[13] In summary, autophagy is required for platelet function at baseline since its complete disruption impairs physiological aggregation. On the other side, in the presence of cardiovascular risk factors, autophagy activation helps to reduce detrimental aggregation. The relevance of platelet autophagy has also emerged in the setting of myocardial ischemia/reperfusion (I/R) injury, a condition where platelets play a pivotal role. In this regard, ischemic preconditioning (IPC) was reported to reduce infarct size in mice undergoing I/R through the activation of platelet mitophagy, the selective form of autophagy for the removal of dysfunctional and senescent mitochondria.[14]

A strong correlation between the reduction of autophagy with increased serum levels of markers of platelet aggregation was also observed in a cohort of patients with atrial fibrillation.[15] Autophagy impairment and platelet aggregation also increase as ageing proceeds.[15] In line with the same evidence, the stimulation of mitophagy, was reported to decrease platelet aggregation in platelets isolated from patients with diabetes, a risk factor for atherosclerosis.[16] This effect was also associated with the reduction of apoptosis and oxidative stress. On the other side, inhibition of mitophagy enhances platelet aggregation.[16] Recently, in a cohort of patients with acute non-ST segment elevation myocardial infarction (NSTEMI), the expression of autophagy-associated protein Beclin1 and LC3II in platelets is reduced compared to healthy subjects. Beclin-1 overexpression *ex vivo* by a lentivirus reduces platelet activation and aggregation in NSTEMI patients.[17] Overall, these results suggest that the restoration of platelet autophagy may represent a suitable therapeutic strategy to reduce cardiovascular complications in subjects at high risk, especially in a subgroup of patients unresponsive to antithrombotic therapy (Table 1). Clinical studies should evaluate the effects *in vivo* of autophagy reactivation in the reduction of thrombotic events in patients at high risk. However, it should also consider the development of potential hemorrhagic episodes since a reduction of platelet aggregation may inevitably predispose to bleeding.

The characterization of the molecular mechanisms underlying the crosstalk between autophagy and platelet aggregation also deserves further studies. Platelet activation increases in the presence of oxidative stress in platelets.[13,15] High levels of H_2O_2 and NADPH oxidase (NOX)-2 activity, two markers of oxidative stress, are associated with an increase in the levels

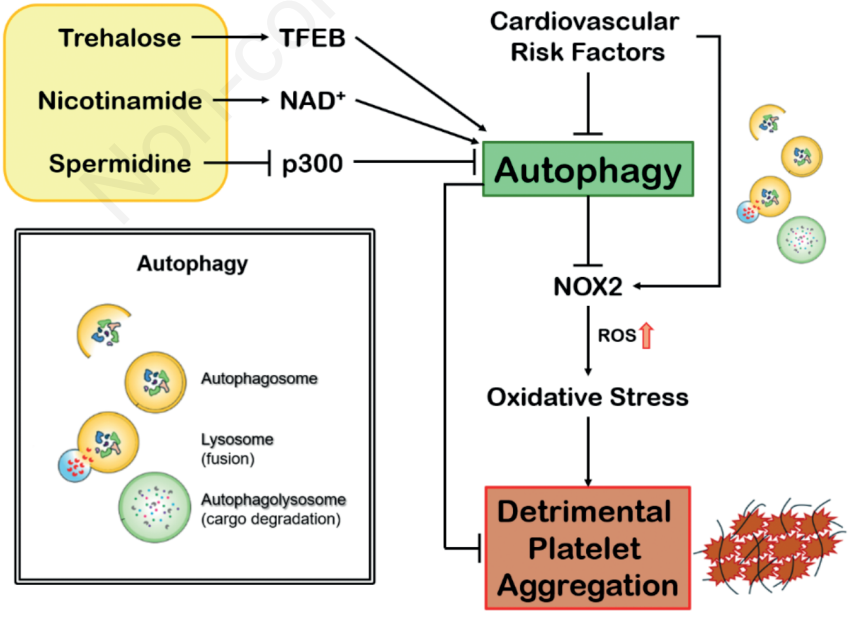

Figure 1. Relevance of autophagy modulation in platelets. In presence of cardiovascular risk factors, platelet autophagy is inhibited. Autophagy activation reduces platelet aggregation. The latter is associated with the reduction of oxidative stress via NOX2 inhibition. Among natural activators of autophagy, trehalose, spermidine and nicotinamide act on multiple molecular mechanisms, which include TFEB, p300 and NAD+. In the lower box are illustrated the different phases of autophagy.

Table 1. The role of autophagy as a therapeutic strategy to reduce platelet aggregation and thrombosis in patients at high risk.

Cohort of patients	Strategy to enhance autophagy/mitophagy	Molecular mechanism	Reference
Metabolic syndrome, atrial fibrillation, smokers	Natural activators of autophagy (spermidine, trehalose, nicotinamide)	Reduction of NOX-2-derived oxidative stress	Carnevale *et al.*[13]
Diabetic patients	Carbonyl cyanide m-chlorophenylhydrazone	Reduction of p53-dependent apoptosis; increase of JNK phosphorylation	Lee *et al.*[16]
Patients with acute non-ST segment elevation myocardial infarction	Beclin 1 overexpression	Not investigated	Pastori *et al.*[18]

of p-selectin and CD40L, known markers of platelet activation, in isolated human platelets.[15] In the specific, NOX-related reactive oxygen species (ROS) production is a strong contributor to platelet activation and platelet-related thrombosis, through different mechanisms that involve NO inactivation, calcium mobilization and F2-isoprostanes formation.[18] Restoration of autophagy in platelet isolated from subjects at high cardiovascular risk also decreases oxidative stress, suggesting that the reduction of oxidative stress may represent the molecular mechanism through which autophagy decreases platelet hyperactivity.[13] In addition to NOX-2, the protective effects of mitophagy induction in platelets of diabetic patients were reported to be associated with the activation of oxidative stress-induced c-Jun N-terminal protein kinases (JNK) pathway and a consequent reduction of p53-induced apoptosis.[16] Further studies should corroborate the involvement of NOX-2 and JNK in relevant animal models of cardiovascular diseases in the presence of pharmacological or genetic inhibition of autophagy. In conclusion, the evidence described here suggests that autophagy is involved in the pathophysiology of platelet function and that the modulation of autophagy in platelets may represent a valid therapeutic option to reduce cardiovascular complications related to platelet hyperactivity.

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