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Protein Degradation Systems in Platelets

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Summary

Protein synthesis and degradation are essential processes that allow cells to survive and adapt to their surrounding milieu. In nucleated cells, the degradation and/or cleavage of proteins is required to eliminate aberrant proteins. Cells also degrade proteins as a mechanism for cell signaling and complex cellular functions. Although the last decade has convincingly shown that platelets synthesize proteins, the roles of protein degradation in these anucleate cytoplasts are less clear. Here we review what is known about protein degradation in platelets placing particular emphasis on the proteasome and the cysteine protease calpain.

Keywords

platelets; protein degradation; proteasome; calpain

Introduction

Numerous studies have now revealed that anucleate platelets use a variety of sophisticated mechanisms to translate mRNA into protein (1–4). Platelets constitutively synthesize actin, GPIIb/IIIa, and likely P-Selectin (5–7) and signal-dependent synthesis generates new proteins important for inflammation, clot retraction, and matrix remodeling (8). In order to maintain protein homeostasis, cells balance protein synthesis with degradation. Protein degradation pathways are well-characterized in nucleated cells and required to eliminate old and misfolded proteins. Protein degradation also generates free amino acids that are recycled into newly-synthesized proteins. In addition, the proteasome is involved in antigen processing, transcriptional control, and apoptosis (9–10). Genetic deletion of key proteasomal subunits is lethal for mice (11) and impairments in proteasomal function have been linked to Alzheimer's, Parkinson's, and other neurodegenerative diseases (9). Despite these critical functions, there is a paucity of research describing protein degradation pathways in anucleate platelets. Below we briefly review our current understanding of protein degradation pathways in platelets.

The Proteasome in Platelets

The proteasome is a large protein complex that consists of a 19S regulatory and a 20S core component. In depth descriptions of the proteasome are described elsewhere (12–14). Next-generation RNA-sequencing demonstrates that platelets express mRNAs for genes known to

be part of the ubiquitin-proteasome pathway (15) and proteomic profiling has shown that platelets possess key proteasomal components (16). Several groups have also detected proteasomal-like activity in platelets (17–22). Yukawa and coworkers were the first to purify proteasome-like activity from the cytoplasmic fraction of platelets. The same group later demonstrated chymotrypsin- and trypsin-like proteasome activity in platelets. Specific proteins, such as c-Mpl, pro-GPIIb and Syk, are also ubiquitinated and degraded in megakaryocytes or platelets by the proteasome (17, 23–24). Cumulatively, these studies strongly suggest that platelets possess an active proteasome.

Even though the proteasome appears to be present, its function(s) in platelets is not yet known. One intriguing possibility is that the proteasome regulates events related to platelet formation and/or the destruction. In this regard, thrombocytopenia is commonly observed when multiple myeloma patients are treated with proteasome inhibitors (e.g. bortezomib) (25). Whether or not bortezomib induces thrombocytopenia by directly inhibiting proteasomal activity in platelets or through in-direct mechanisms is unclear. An argument for the former is that bortezomib inhibits platelet aggregation *in vitro* (26). Recent work from Nayak and colleagues also demonstrates that the calcium ionophore A23187 augments proteasome activity in platelets (18). This suggests that the platelet proteasome is responsive to environmental cues and, in particular, changes in intracellular calcium levels. Consistent with this notion, endogenous activators of the platelet proteasome have been identified (19–22). Initially, Yukawa and colleagues purified a 170kDa polypeptide complex that activated chymotrypsin- and trypsin-like catalytic subunits of the proteasome (21–22). Later the proteasome activator PA28, which activates the chymotryptic activity of the proteasome, was discovered in platelets (19). PA28 had previously been shown to modulate antigen peptide production by the proteasome in other cells (27).

Necchi and coworkers found that platelets from patients with ANKRD26-related thrombocytopenia contain intracellular vesicles that are enriched for proteasome-like structures and ubiquitinated proteins, which they referred to as ubiquitin/proteasome-rich particulate cytoplasmic structures (PaCSs) (28). In addition to decreased platelet counts, patients with this syndrome have compromised platelet aggregatory responses, leukocyte adhesion, and are at increased risk for developing cancer. Furthermore, the proteasome proteins PSB8 (29) and PSB1 (30) are decreased in platelets from patients with coronary artery disease and acute myocardial infarction. In contrast, our group has observed increased expression of proteasome subunits and proteasomal activity in platelets isolated from patients diagnosed with sepsis (unpublished observations). These reports suggest that acute and chronic diseases alter proteasome activity in platelets. It remains to be determined if alterations in platelet proteasome activity regulates the development of disease or is a consequence of the disease process. Regardless, these observations suggest that the proteasome is differentially regulated in human disease and protein degradation pathways affect platelet function.

Functional role of Calpain in Platelets

Calpains are calcium-dependent, lysosomal cysteine proteases that are rapidly activated by stimuli that induce intracellular calcium fluxes, including calcium ionophores, pore forming toxins such as shiga-toxin (31), streptolysin (32) or porB (33), and thrombin (34). Talin (35), filamin, fodrin (36), vinculin (37), kindlin-3 (38), and myosin-light chain kinase (39) are well known substrates of calpain. Of note, these substrates are critical cytoskeletal and membrane proteins, which may explain why calpain activation in platelets regulates granule secretion and cell spreading (40) and genetic deletion of μ -calpain attenuates platelet aggregation and clot retraction (41). Microparticle shedding is similarly linked to calpain activity in platelets (42).

Because calpains display widespread proteolytic activity, they regulate a variety of diseases (43). Platelet dysfunction in diabetes results in increased calpain activity and subsequent cleavage of septin-5, which promotes the release of CCL5 and TGF- β from α -granules (44). Thus, calpain activation in platelets induces the release of atherosclerosis promoting cytokines that effect vascular responses in diabetes patients. Through cleavage of SNAP-23, calpain also regulates local release of α -granular constituents at areas of thrombus formation (45) and calpain-dependent proteolysis of vWF promotes platelet aggregation in thrombocytopenic patients (46).

Calpains have central roles in cell death pathways and emerging evidence demonstrates that anucleate platelets undergo apoptosis. The apoptotic cascade is a classic example of how protein cleavage triggers enzymatic activation of downstream proteases that drive intracellular signaling events. Calpains are capable of enzymatically cleaving pro-caspases such as caspase-7 (47) or caspase-12 (48) into their active forms. Calpains also cleave the anti-apoptotic protein Bcl-x_L (49). We recently demonstrated that Bcl-x_L undergoes calpain-mediated degradation in human platelets (50). Bcl-x_L degradation is induced by bacteria or calcium ionophores and rescued by specific calpain inhibitors. Degradation is more rapid and robust when platelets are exposed to bacterial strains that secrete toxins. In this regard, non-virulent *E. coli* gained degrading properties when they were forced to express α -hemolysin. Conversely, genetic deletion of α -hemolysin in virulent *E. coli* strains abrogated cleavage of Bcl-x_L. These observations suggest that calpain-dependent cleavage of Bcl-x_L, a pro-survival protein that enhances survival in platelets (51), contributes to thrombocytopenia that is commonly observed in patients with bacterial sepsis.

We also found that traditional platelet agonists such as thrombin or platelet-activating factor (PAF) do not induce cleavage of Bcl-x_L. This is surprising because thrombin activates calpain in platelets (34). One simple explanation is that pore-forming bacteria and calcium ionophores induce greater calcium fluxes than thrombin or PAF. Whatever the mechanism, these data indicate that platelet activation and apoptosis are distinct processes (52–53).

Conclusion

Despite their anucleate stature, there is a growing appreciation that platelets have complex biosynthetic and degradation systems. Understanding how these systems counterbalance one another will shed considerable insight into platelet function in health and disease. In regards to degradation, it is clear that platelets possess at least two protein degradation pathways (Figure 1): the proteasome, which seems critical for steady and gradual degradation of ubiquitinated proteins that may accumulate as platelets age or are exposed to chronic environmental challenges such as the diabetic milieu; and, calpain-dependent degradation that mediates cleavage of a defined set of substrates that modulate rapid cell signaling events in platelets (54). Although the responsible pathway(s) needs to be determined, preliminary results from our group demonstrate that protein degradation is widespread in calcium-ionophore treated platelets (Figure 2). As we move forward, it will be important to identify the complete repertoire of degraded proteins in stimulated platelets, the signals that induce degradation, and determine how these degradation processes regulate platelet function.

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Figure 1. Protein degradation pathways in human platelets

The schematic highlights potential functions of the proteasome and calpain protein degradation pathways in human platelets. The proteasome degrades polyubiquitinated (Ub) proteins that have been marked for degradation. Calcium-dependent activation of calpain cleaves a variety of intracellular proteins including Bcl-x_L which is followed by translocation of pro-apoptotic bax into mitochondria and platelet apoptosis. Interplay between the calpain and proteasome degradation pathways has also been observed.

Figure 2. Calcium-dependent protein degradation in human platelets

Platelets were left alone (A) or stimulated with A23187 (B) for 1 hour and intracellular proteins were separated by 2-D gel electrophoresis. The white circles identify proteins that disappear after calcium ionophore stimulation.