

# Platelet granules – secretory and secretive

Kerstin Jurk

Center for Thrombosis and Hemostasis (CTH), University Medical Center, Mainz, Germany

## Keywords

platelets, secretory granules, storage pool disease

## Summary

The article reviews three recent publications addressing physiological and pathological aspects of platelet granules and release as well as limitations of recent screening tests for diagnosis of non-syndromic inherited  $\delta$ -storage pool disease (1-3).

## Korrespondenzadresse

PD Dr. rer. nat. Kerstin Jurk  
Center for Thrombosis and Hemostasis (CTH)  
University Medical Center Mainz  
Langenbeckstr. 1, 55131 Mainz, Germany  
E-Mail: kerstin.jurk@unimedizin-mainz.de

## Schlüsselwörter

Thrombozyten, sekretorische Granula, Storage-Pool-Erkrankung

## Zusammenfassung

Der Beitrag gibt eine Übersicht über drei aktuelle Publikationen zu physiologischen und pathologischen Aspekten von Thrombozyten-Granula und -Freisetzung sowie den Limitationen der Screening-Tests zur Diagnose von nicht-syndromalen erblichen  $\delta$ -Storage-Pool-Erkrankungen (1-3).

## Plättchen-Granula – sekretorisch und geheimnisvoll

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In response to injury activated platelets release a bulk of proteins and small biomolecules from the storage pool of different granules in a coordinated manner. Fusion of granule membranes with the plasma membrane and membrane of the surface-connected or open canalicular system (OCS) leads to secretion of granule cargos, which facilitate platelet aggregation and platelet-based coagulation, thereby contributing crucially to haemostatic plug and thrombus formation.

Beside their role in haemostasis and thrombosis, secreted granule contents contribute to wound healing, immune response and inflammation and they are involved in pathologic processes of

- arteriosclerosis,
- infection,
- cancer and
- metastasis (4).

Inherited or acquired platelet granule disorders are frequently associated with mild

to moderate bleeding diathesis, which may occur as life-threatening complications upon surgery or trauma. Platelet storage pool diseases (SPD) are characterised by defects of granule secretion or by defects of granule biogenesis, e.g. decreased/absent numbers or content of  $\alpha$ -granules ( $\alpha$ -SPD),  $\delta$ -granules ( $\delta$ -SPD) or both ( $\alpha$ - $\delta$ -SPD).

They can present as isolated or syndromic forms, i.e. in association with other disease manifestations, with normal or altered platelet count and volume (4–6).

## Platelet granules and release – update needed

Classically, three prominent granule types are described in platelets:

- $\alpha$ -granules,
- $\delta$ -granules and
- lysosomes.

$\delta$ -granules, which are highly electron dense (► Fig. 1) secrete ADP, ATP, polyphosphates,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  cations, serotonin and histamine, thereby amplifying platelet aggregation and platelet-dependent coagulation.

Activated platelets release distinct acid glycohydrolases, cathepsins and heparinase from lysosomes, which contribute to extracellular matrix degradation and cell migration.

Platelet  $\alpha$ -granules are most abundant (► Fig. 1) and packed with multifunctional proteins and peptides, synthesised in megakaryocytes or endocytosed from plasma. In addition to adhesion proteins such as fibrinogen and von Willebrand factor,  $\alpha$ -granules contain factors with supporting or inhibiting functions of coagulation, fibrinolysis and angiogenesis.

There is increasing evidence that  $\alpha$ -granules are not equal, but rather heterogeneous in cargo packing and/or secretion kinetics.

Recently, Crescente and co-workers identified a potential novel granule-independent release mechanism of the thiol isomerases protein disulphide isomerase (PDI) and endoplasmic reticulum protein 57 (ERp57) (1). In resting platelets, PDI and ERp57 represent important endoplasmic reticular chaperones and are important surface-located regulators of platelet activation. The authors of this study demonstrated that PDI and ERp57 are mobilised to the surface of activated platelets through a dense tubular membrane system near the inner surface of the plasma membrane in an actin-polymerisation-dependent but secretory-membrane-fusion-independent manner.

This study suggests a new model of different platelet compartments that enable protein release onto the platelet surface: On

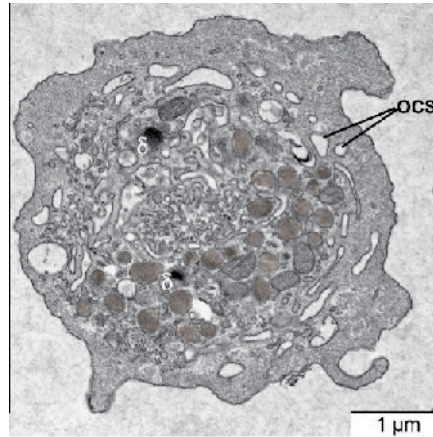
the one hand, secretory proteins are packed into granules when megakaryocytes form pro-platelets. On the other hand, distinct proteins from megakaryocytic endoplasmic reticulum move to developing membranes of the dense tubular system and localise adjacent to the surface membrane of forming platelets.

However, the molecular mechanisms of differential protein trafficking need to be further characterised to classify new platelet granule types and subtypes as well as other platelet release compartments.

## Inherited disorders of $\alpha$ -platelet-granule formation = Grey platelet syndrome?

Originally, grey platelet syndrome (GPS) has been identified as autosomal recessive inherited disorder of  $\alpha$ -granule biogenesis caused by mutations in *NBEAL2* coding for the vesicle trafficking relevant neurobeachin-like 2 (NBEAL-2) protein (7). Platelets from patients with GPS are enlarged and appear “grey” in blood smears due to  $\alpha$ -granule deficiency. In addition to the bleeding tendency, myelofibrosis and splenomegaly are frequently associated with GPS. However, distinct mutations in the transcription factor genes growth-factor-independence-1b (GFI1B) and GATA1 are also known to cause platelet  $\alpha$ -granule deficiency associated with large platelets, but with autosomal dominant and X-linked inheritance, respectively (8).

Based on the increasing use of high throughput sequencing to identify further novel genes involved in  $\alpha$ -granule biogenesis, the question arises whether GPS is caused by different gene defects of  $\alpha$ -granules. A recent study by E. Turro and co-workers nicely demonstrated that a gain-of-function mutation in the proto-oncogene tyrosine-protein-kinase-SRC-gene causes a GPS-like disorder but not classical GPS (2). The authors studied a three-generation pedigree including 9 affected members with a dominant inheritance of bleeding associated with thrombocytopenia,



**Fig. 1** Thin-section transmission electron image of a human platelet. Platelets from a healthy donor were prepared at the Center for Thrombosis and Hemostasis in Mainz and analysed by transmission electron microscopy in M. Schaller's core facility, University Department of Dermatology in Tübingen. Abundant  $\alpha$ -granules are coloured in brown. Sparser  $\delta$ -granules are indicated as  $\delta$ . Lysosomes are not detected. OCS: surface-connected or open canalicular system. Scale bar 1  $\mu$ m.

nia, myelofibrosis and bone pathologies with facial dysmorphism and premature edentulism.

Using genome sequencing and human phenotype ontology, the authors identified a E527K substitution of SRC that causes increased SRC activity leading to enhanced overall tyrosine phosphorylation especially in patients' megakaryocytes and platelets. SRC is involved in platelet signalling pathways mediated by

- integrins  $\alpha_{IIb}\beta_3$  and  $\alpha_2\beta_1$ ,
- Gq-coupled thrombin protease-activated receptors 1 and 4,
- Gi-coupled ADP receptor P2Y<sub>12</sub> and
- von Willebrand factor receptor GPIIb.

The study showed that the E527K variant of SRC results in defective pro-platelet formation and more immature megakaryocytes. In addition, E527K-transduced megakaryocytes presented with altered organisation of the actin cytoskeleton, which might contribute to the  $\alpha$ -granule defect. Although patients' platelets showed a GPS-like phenotype (e.g.  $\alpha$ -granule paucity, decreased  $\alpha$ -granule storage pool, abundant OCS-like vacuoles and dysfunction), the presence of both unusually large

and small platelets without any type of granules and lacking internal membranes is not typical for classical GPS. It remains exciting to see which further genes, so far not known or expected to be involved in megakaryopoiesis, will regulate  $\alpha$ -granule biogenesis.

## Non-syndromic forms of inherited $\delta$ -platelet-granule defects – often missed by routine screening tests

In contrast to the syndromic Hermansky-Pudlak or Chediak-Higashi syndromes, isolated inherited platelet  $\delta$ -granule disorders are much more common than appreciated. The analyses of stained blood smears and light transmission aggregometry (LTA) are recommended as first-step tests for diagnosis of SPD and are available in most clinical centres.

However,  $\delta$ -granule defects cannot be detected by examination of blood smears. In case of suspected  $\delta$ -SPD, impaired secondary wave aggregation in response to epinephrine and ADP and reduced collagen response are expected but not always present. Indeed, LTA may not be sensitive for  $\delta$ -SPD and therefore complex analyses of platelet granule secretion is still necessary (9), but usually restricted to specialised centres.

In addition to platelet phenotyping, next-generation sequencing is recommended to complement the diagnosis of inherited platelet function and number disorders.

The ThromboGenomics Consortium published recently that targeted high throughput sequencing of 63 genes involved in bleeding, thrombotic and platelet disorders is a sensitive genomic approach to validate pathogenic variants in patients with suspected aetiology, such as

- Glanzmann thrombasthenia,
- May-Hegglin disorder or
- syndromic platelet  $\delta$ -granule defects, e.g. Hermansky-Pudlak syndrome (3).

In contrast, pathogenic variants in patients with bleeding diathesis, but with uncertain aetiology based on normal coagulation or platelet function tests and those with abnormal platelet function tests indicating SPD, were not frequently detected. One pathogenic variant in *TUBB1* as well as in *MYH9* and two cases with mutations in *RUNX1* could be detected in this group of 62 unrelated patients with unclear bleeding disorder. This sequencing platform targets 36 platelet disorder-related genes, but only a few genes, which are known to be associated with non-syndromic platelet-granule secretion defects, such as *NBEA*, *RUNX1* and *FLI1* (10), were included. This targeted sequencing study demonstrates its current limitation for diagnosis of isolated  $\delta$ -SPD.

### Conflict of interest

The author declares that there is no conflict of interest.

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