POST-ISTH: Novità dal meeting di Toronto 2015

Piastrine: fisiopatologia

Erica De Candia

Università Cattolica -Roma

Fondazione Policlinico Universitario A. Gemelli
Università Cattolica del Sacro Cuore

Novità ISTH 2015: Piastrine: Fisiopatologia

i) Alternative platelet function throughout the body
ii) Traditional hemostatic platelet function
iii) Making platelets for transfusion

I. Alternative Platelet Function throughout the Body

- Inflammation
- Infection
- Cancer
- Platelets as carrier cells

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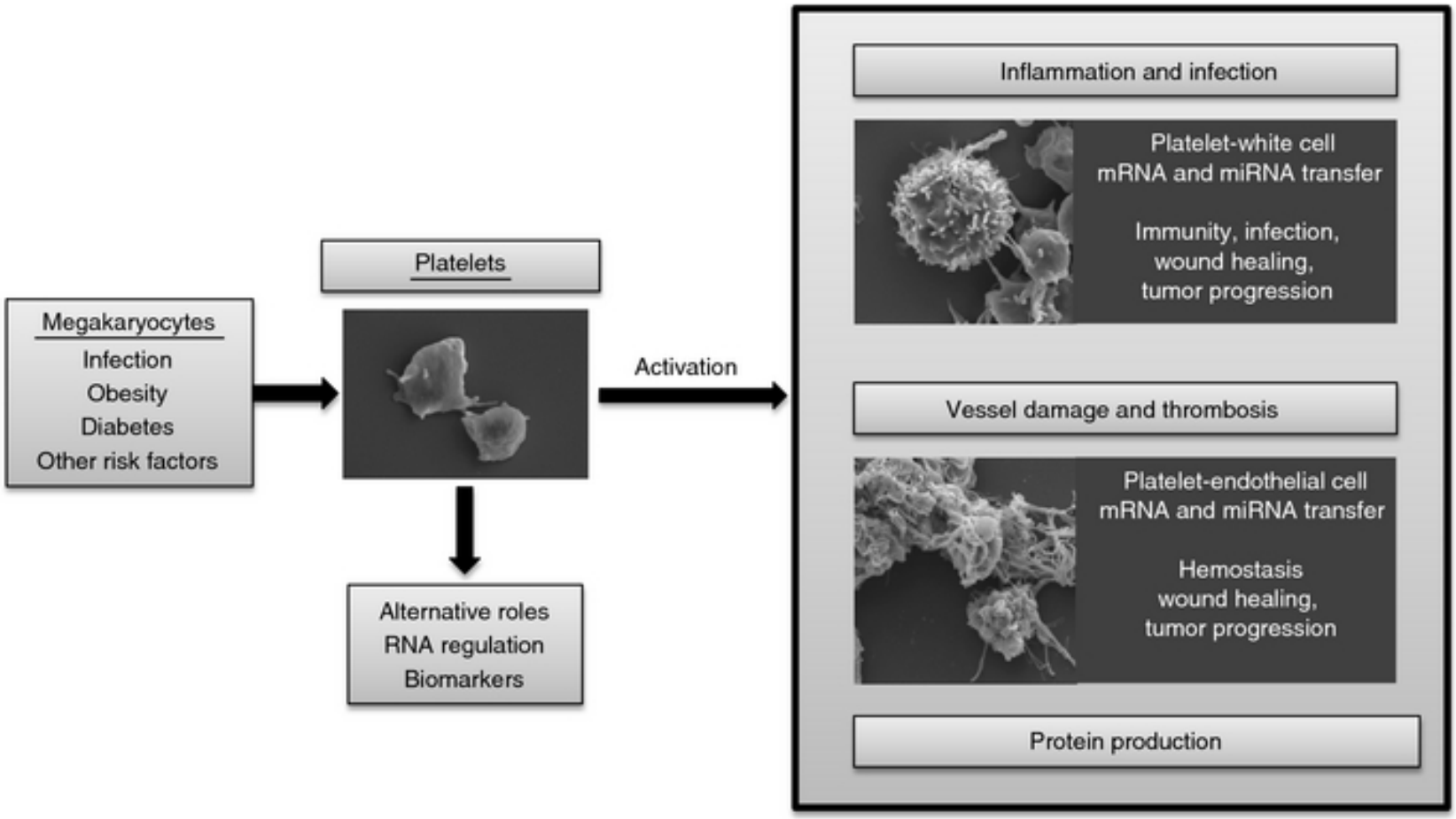
The Role of Circulating Platelet Transcripts



L. Clancy and J.E. Freedman
Journal of Thrombosis and Haemostasis
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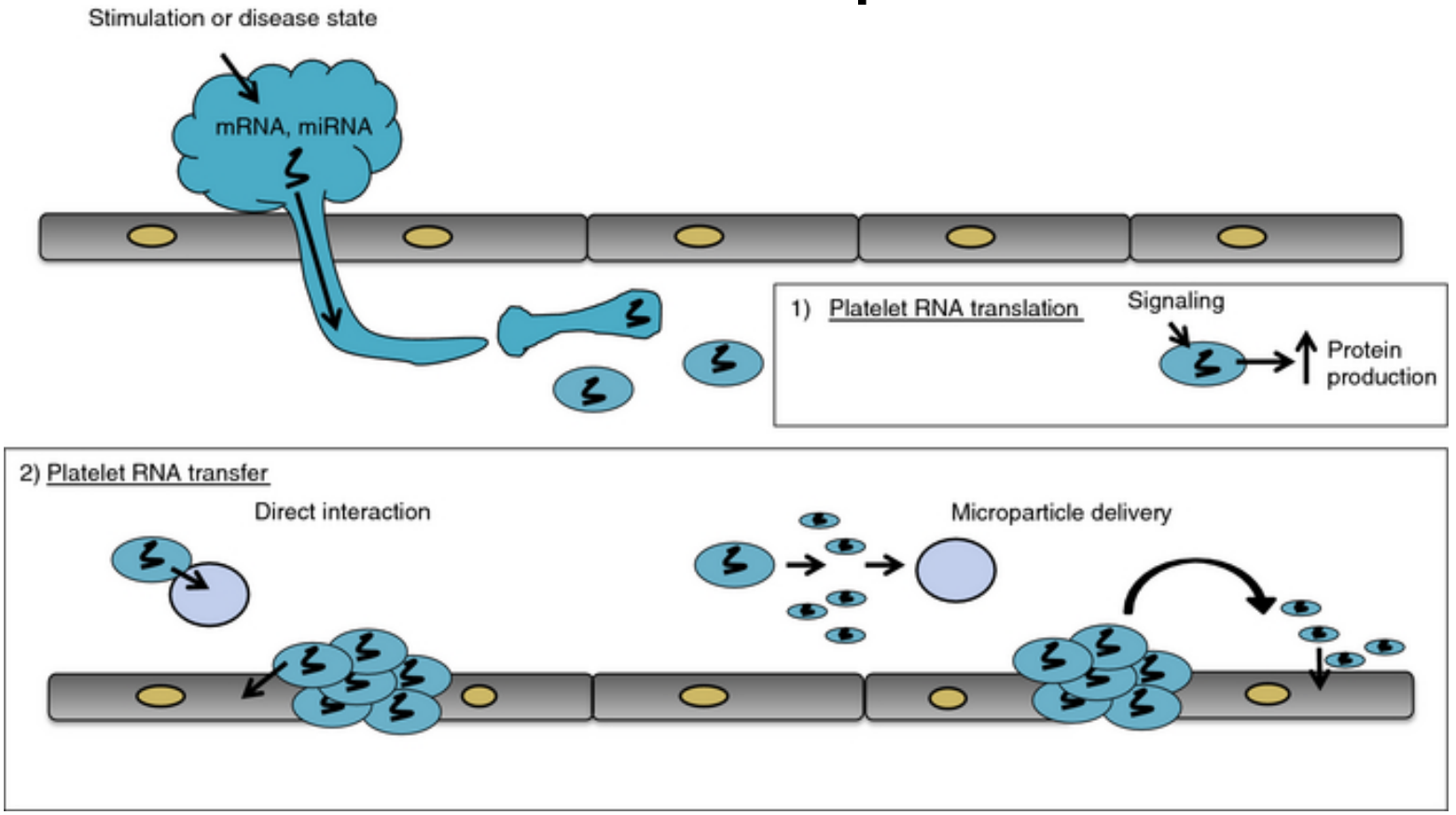

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Protective Effects of Platelets on Vascular Integrity Differ between Organs

Platelet granule release is crucial for vascular integrity in the ischemic brain but not in the inflamed skin or lung

Deppermann C[1], Kraft P[2], Wolf K[1], Nurden P[3], Stoll G[2], Stegner D[1] and Nieswandt B[1]

Mice deficient for both NBEAL2 and Munc13-4 lack alpha-granules and cannot secrete their dense granule content.

These mice have impaired aggregation, prolonged bleeding times and defective arterial thrombus formation *in vivo.*

In models of skin and lung inflammation there were no hemorrhage.
In a model of thrombo-inflammatory brain infarction vascular integrity was impaired.

The mechanism by which platelets maintain vascular integrity differ between organs.

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Thrombus formation caused by invading pathogens: the role of platelets

Kerrigan SW

in maintaining thrombosis and haemostasis, platelets also play a critical role in orchestrating the immune response. Being the first cell at the site of injury they are perfectly placed to assess the extent of the damage and recruit immune cells as is necessary. As a first line of defence platelets can act as primitive immune cells themselves by interacting with invading pathogens. Unlike physiological platelet agonists that confer specificity for a platelet receptor, bacteria can engage several platelet receptors at the same time. Binding maybe as a result of a direct interaction when a bacterial adhesin binds directly to a platelet receptor or other surface expressed component on the platelet. Alternatively an indirect interaction occurs when a bacterial adhesin binds to a plasma protein or other soluble elements of the immune system such as immunoglobulins and complement proteins which bridge the bacteria to a specific receptor or other expressed component on the platelet surface. Either way the end result is platelet activation. A

Platelet pathways involved in bacterial-platelet-leukocyte interactions: CLEC-2, TLR4, TLR9, *myeloid differentiation primary response* 88 (MYD88)

Direct bacteria-platelet interaction

CLEC-2 is required for the activation of mouse platelets by bacterial DNA mimetics

Delierneux C[1], Hego A[1], Lecut C[1], Vandereyken M[2], Musumeci L[2], Rahmouni S[2], Bours V[3], Lancellotti P[1,4] and Oury C[1]

Oligonucleotides mimicking bacterial DNA displaying potent immunostimulatory activity (CpgODNs) were able to activate platelets from wt and TLR9- and MyD88-deficient mice and to promote fibrin generation and thrombus formation *in vivo*.

Only CLEC-2 deficiency abolished CpG ODN induced platelet activation and aggregation, showing that CLEC-2 may play an important role in the interplay between platelet and immunity

Platelet pathways involved in bacterial-platelet-leukocyte interactions: CLEC-2, TLR4, TLR9, MYD88

Effect of leukocyte-platelet interaction on bacteria

Platelet Myd88 enhances cytokine production induced by TLR agonists and *Klebsiella pneumoniae*

Claushuis TA[1,2], de Stoppelaar SF[1,2], van 't Veer C[1,2] and van der Poll T[1,2,3]

MYD88 is the adaptor protein of TLR4 and TLR9 in platelets, both receptors essential in host defense to *K.Pneumoniae.* Platelets modulate cytokine responses by leukocytes through not fully elucidated mechanisms .

Platelet specific MyD88 deficient mice were generated, the effects of LPS (TLR4 agonist) and CpG (TLR9 agonist) stimulation on production of cytokines in whole blood in vitro was measured.

Platelet specific MyD88 deficient mice produced significantly less TNF compared with control littermates.

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Pletalets in cancer progression and metastasis

Byzova TV and Kerr B

There is an increasing body of experimental and clinical evidence that platelets and hemostatic system in general directly influence tumor growth and, possibly, tumor metastasis. We show that distal tumors

Tumors secrete a number of factors which can be sequestered by platelets and released upon platelet activation. Several tumor-derived factors are present exclusively in platelets but not in plasma of tumor-bearing animals. Platelets of tumor-bearing animals contain high amounts of factors controlling bone metabolism and, potentially, bone metastasis. TGF-β1, known to promote skeletal metastasis of several cancers, is present at the highest concentration in platelets and the con-

Platelets and cancer

CLEC-2 facilitates hematogenous tumor metastasis and *in vitro* tumor growth, but not *in vivo* tumor growth

Shirai T[1], Inoue O[2], Hirayama K[3], Endo H[4], Sato-Utida H[5], Fujii H[3], Suzuki-Inoue K[1] and Ozaki Y[1]

Identification of a novel C-type lectin-like receptor 2 inhibitor that suppresses podoplanin-induced platelet aggregation and cancer metastasis

Chang Y-W[1], Hsieh P-W[2], Cheng J-C[3] and Tseng C-P[1,4,5]

15(S)-HETE is a pro-angiogenic factor produced by platelets through COX-1

Rauzi F[1,2], Kirkby N[1], Edin M[3], Zeldin D[3], Whiteford J[2], Mitchell J[1] and Warner T[2]

RNA Signatures in Platelets from Cancer Patients

Wurdinger T.

Platelets are a source for the non-invasive monitoring of cancer biomarkers

- Blood platelets contain tumor-derived RNA biomarkers. Blood, 2011
- Transcription profiling in human platelets reveals LRRFIP1 as a novel protein regulating platelet function. Blood, 2010
- Rearranged EML4-ALK fusion transcripts sequester in circulating blood platelets and enable blood-based crizotinib response monitoring in non-small-cell lung cancer. Oncotarget, 2015

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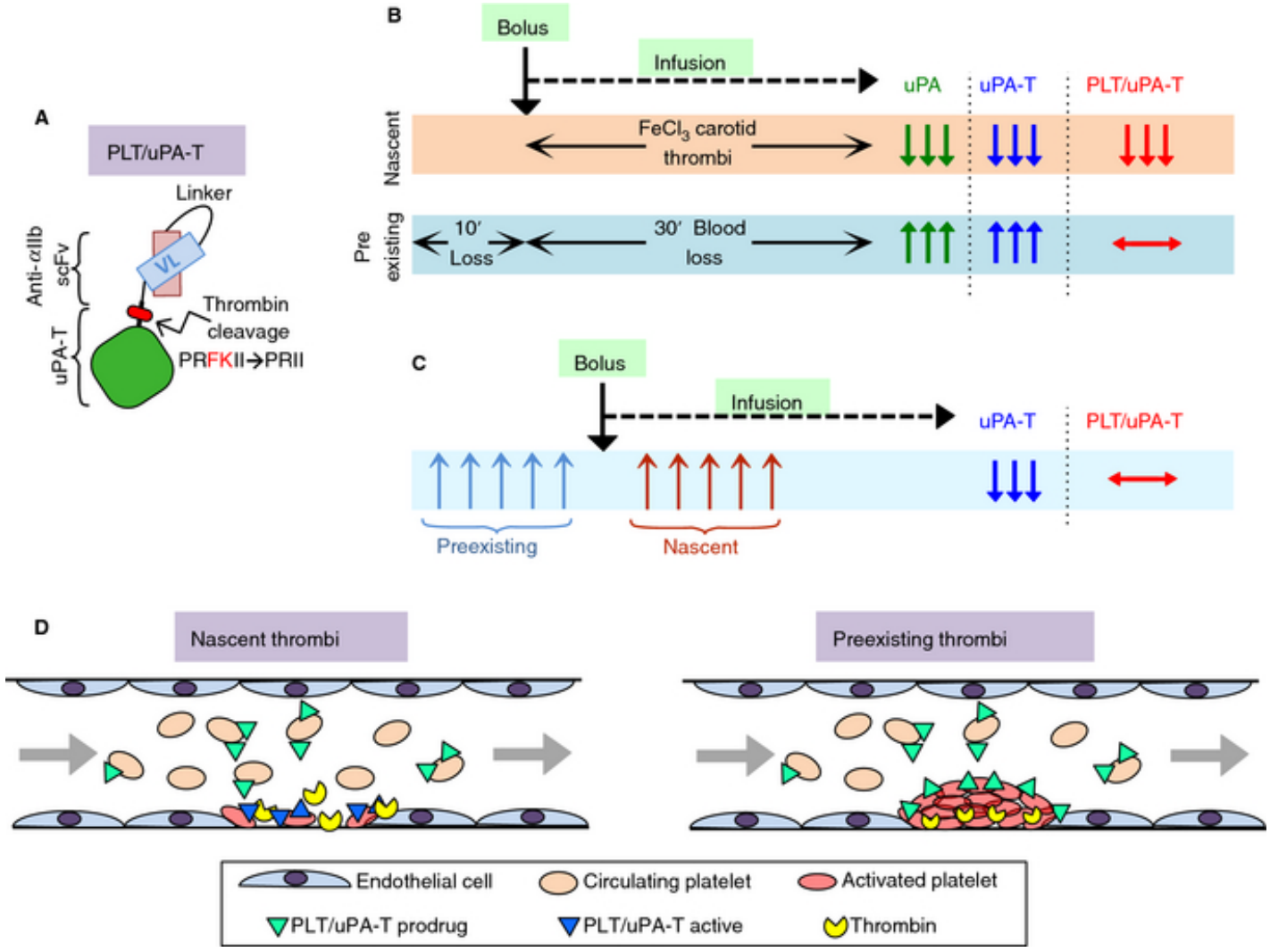
Platelets as “Carrier” Cells

Platelet-delivered therapeutics

R. LYDE,*† D. SABATINO,*‡ S. K. SULLIVAN§ and M. PONCZ*‡

1. uptake of protein of interest by platelets and storage in alpha-granules (like FV)
2. Construction of chimeric proteins that bind to a unique platelet surface receptor

Platelet-delivered therapeutics



Journal of Thrombosis and Haemostasis
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Engineering platelets for the delivery of RNA

Novakowski S[1,2], Chan V[1,2], Law S[1] and Kastrup C[1,2]

Nanoliposomes are used to introduce RNA to platelets for the release and delivery to other cell types following platelet activation.

Platelets uptake nanoliposomes by endocytosis and the RNA is released during platelet activation.

Platelets can be created to deliver miRNA and RNA against mediators of inflammation at the site of atherosclerotic plaques.

Transcription of exogenous RNA in human platelets

Chan VWT[1,2], Novakowski S[1,2], Law S[1] and Kastrup C[1,2]

Reporter mRNA are transcribed within liposomes, that are internalized by human platelets *ex vivo*

Platelets can be created to deliver miRNA and RNA for therapeutic use

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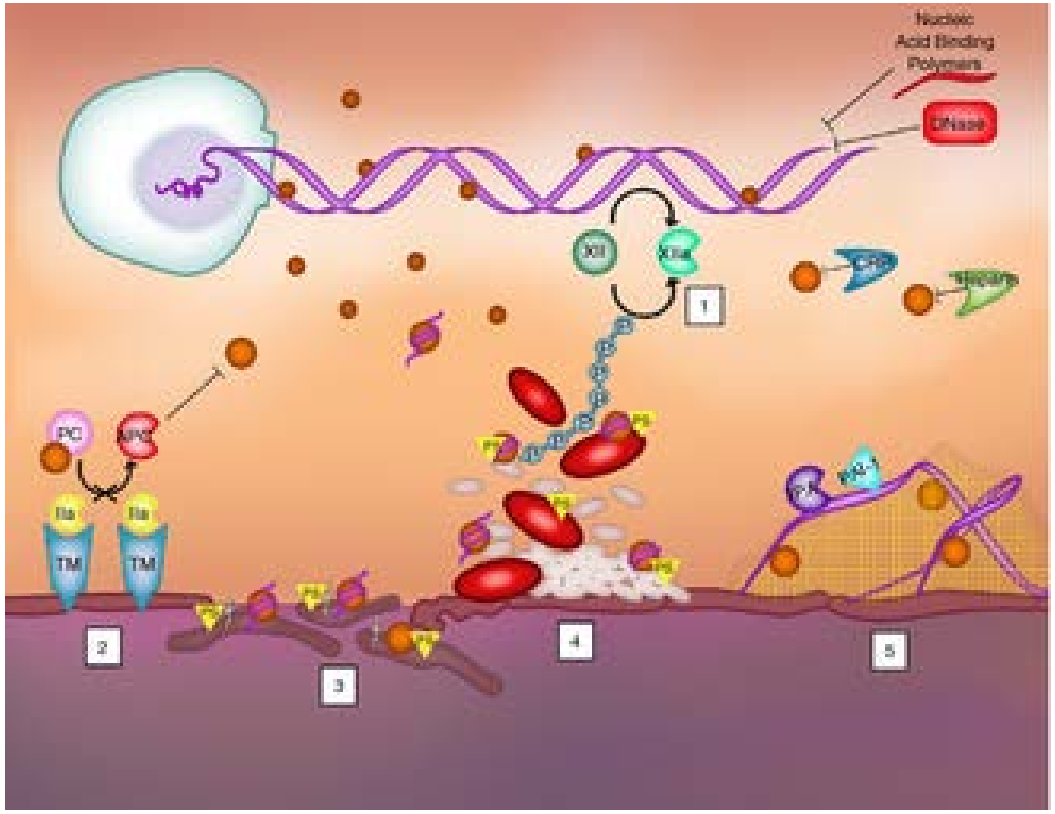
Extracellular DNA and Histones

- The existence of extracellular DNA in human plasma, also known as cell-free DNA (cfDNA), was first described in the 1940s. In recent years, there has been a resurgence of interest in the functional significance of cfDNA, particularly in the context of neutrophil extracellular traps (NETs).

- cfDNA and histones are key components of NETs that aid in the host response to infection and inflammation. However, cfDNA and histones may also exert harmful effects by triggering coagulation, inflammation, and cell death and by impairing fibrinolysis.

Extracellular DNA and Histones: Double-Edged Swords in Immunothrombosis



TJ Gould, Z Lysov, PC Liaw
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Histones as New Platelet Agonists?

Histone-associated thrombocytopenia: a new cause of thrombocytopenia in critically ill patients

Alhamdi Y[1], Abrams ST[1], Welters I[2], Wang G[1] and Toh C-H[1,3]

- Thrombocytopenia is common in critically ill patients and is independent predictor of prolonged ICU stay and mortality.
- Extracellular histones could induce thrombocytopenia when injected into experimental mice.
- A prospective study on 160 ICU patients showed a significant inverse correlation between histone levels on admission and plt count in 2nd and 3rd ICU days.
- Plasma from ICU patients was able to induce aggregation of freshly isolated control platelets, abolished by ant-histone antibodies.

- Neutralizing histones as a new strategy to treat thrombocytopenia in critical ill patients?

Histones as New Platelet Agonists?

The impact of circulating histones on platelet activation and thrombus formation *in vivo*

Stalker TJ, Wu J and Brass LF

Background: Circulating histone levels are elevated in the setting of trauma and correlate with the severity of injury. Studies have suggested that histone toxicity may contribute to tissue damage, as well as the development of a coagulopathic state in a subset of trauma patients. In mouse models, histone infusion leads to thrombocytopenia, elevated cytokine levels, tissue damage and death.
Aims: In the present study, we sought to determine the impact of circulating histones on platelet function and thrombus formation *in vivo*.

Infusion of high doses of histones in vivo to mice results in activation of a subpopulation of circulating platelets and increased thrombus stability, in a model of laser-induced vascular injury in cremaster arterioles

Tissue Factor Induced Platelet Aggregation

Considerable variability of platelet response to tissue factor in healthy individuals and potential role of fxa inhibitor as an anti-platelet agent

Murasaki KM[1], Ishigaki K[2] and Hagiwara N[1]

- Measurement of platelet-activation specific antigens using flow cytometry analysis after TF stimulation.
- Activation of platelets occurred within 2 minutes and it was dose dependent.
- Using Rivaroxaban as inhibitor of thrombin generation prevented TF-induced platelet activation.

- Blocking coagulation factors could have a role as anti-platelet agent?

Inhibiting the Platelet Procoagulant Activity as a New Antithrombotic Strategy?

Novel peptide and peptidomimetic inhibitors of prothrombinase assembly on phosphatidylserine inhibit thrombin generation and fibrin formation

Kastelowitz N[1], Jarvis A[2], White OR[1], Brown PN[1], Brodsky GL[2], Tamura R[1], Di Paola JA[2] and Yin H[1]

- Probes for phosphatidyl serine (PS) were developed to antagonize coagulation factors/lipid membrane association.
- By using surface plasmon resonance method coagulation factors/PS binding was shown to be inhibited.
- PS probes inhibited the enzymatic activity of prothrombinase in the presence of activated platelets.

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Making Platelets *ex vivo* for Transfusion

- Platelets from donors are of limited supply
- Platelets from donors can be preserved for 5 days at room temperature
- Risk of bacterial contamination
- Large waste of platelets
- Need for alternative sources for platelets

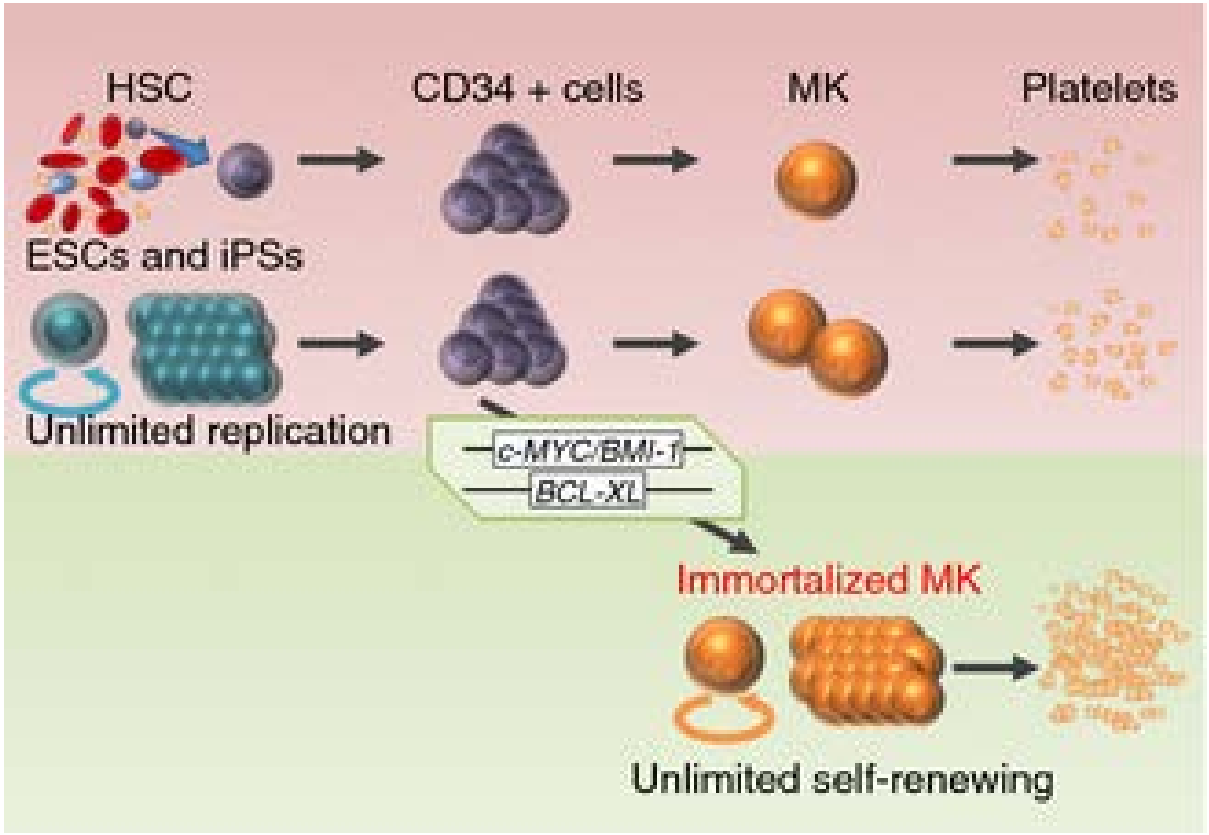
Making Platelets *ex vivo* for Transfusion

- 1 unit of platelets for transfusion contains about 500 billion platelets.
- Current ex vivo technique generate about 100 plts/MK

Ex vivo platelet generation has two major obstacles:

1) Expansion of CD34+ cells into megakaryocytes
2) Shedding of platelets from MKS

Manipulating Megakaryocytes to Manufacture Platelets *ex vivo*



P Karagiannis and K.Eto
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Recapitulating the Microenvironment to Enhance Platelet Production *ex vivo*

- 2 to 3 dimensions bioreactors
- Coating the scaffold with TPO and fibronectin
- Bioreactors made of silk microtubes coated with extracellular matrix and growth factors
- Adding shear stress generated by parallel flows running around the scaffolds (microfluidic bioreactor)

A microfluidic chamber for elongating megakaryocytes and producing functional platelets

Blin A[1], Goff AL[2], Poirault-Chassac S[1,3], Sicot G[1], Teste B[2], Magniez A[1], Reyssat M[2] and Baruch D[1,3]

[1]PlatOD; [2]CNRS UMR 7083 Gulliver, ESPCI; [3]UMR_S1140, Inserm, Paris, France

Aims: To build a microfluidic system where MK are directly injected in the chamber at a high shear allowing for their capture, subsequent elongation, fragmentation into (pro)platelets, and rapid collection of newly formed platelets for their characterization.

Methods: The chamber is made of a rectangular microchannel with an array of VWF-coated micropillars used as anchors for MK. 2 large

Time lapse observations reveal that the fragments released by a single anchored MK can represent 350 platelets in terms of volume.

Conclusion: This chamber allows high MK throughput and rapid formation of platelets with preserved functions, opening up the possibility of platelet production for therapeutic use.

GRAZIE PER L'ATTENZIONE