Piastrine: genoma e trascrittoma

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"Workshop post-ISTH: novità dal meeting di Toronto 2015"
Bergamo, 29-30 gennaio 2016

Topics

1. Genetic regulation of platelet function in health and disease

- Genetic regulation of megakaryocytes and platelets

  Andy Weyrich (State of the art)

- Dicer, the key enzyme of RNA interference, is regulated by thrombin stimulation in human platelets

  Manni G et al. (Oral comunication, Young Investigator Award)

- Diphosphorylated lipid A triggers tissue factor mRNA splicing in human platelets

  Krauel K et al. (Oral comunication)

2. Platelet mRNAs and miRNAs in the interaction with other cells

- The role of circulating platelet transcripts

  Jane Freedman (State of the art)

- RNA signatures in platelets from cancer patients

  Thomas Wurdinger (SSC Platelet Physiology, lecture)

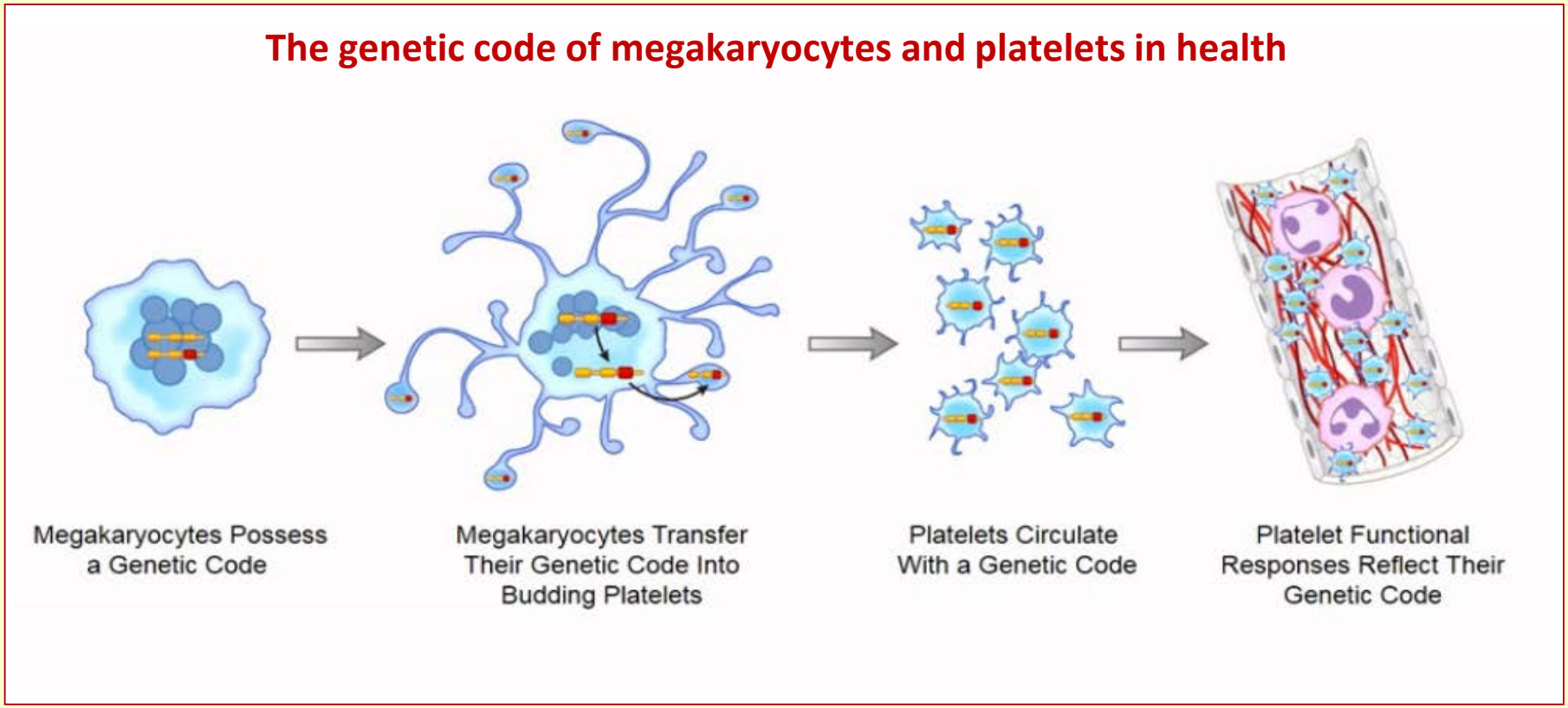
- Platelets release extracellular vescicles in an agonist dependent manner but release a consistent profile of microRNA

  Ambrose AR et al. (Oral comunication, Young Investigator Award)

Genetic regulation of megakaryocytes and platelets

Andrew S. Weyrich

State of the art lecture



The transfer of genetic information in the form of coding mRNAs and regulatory miRNAs affects protein expression

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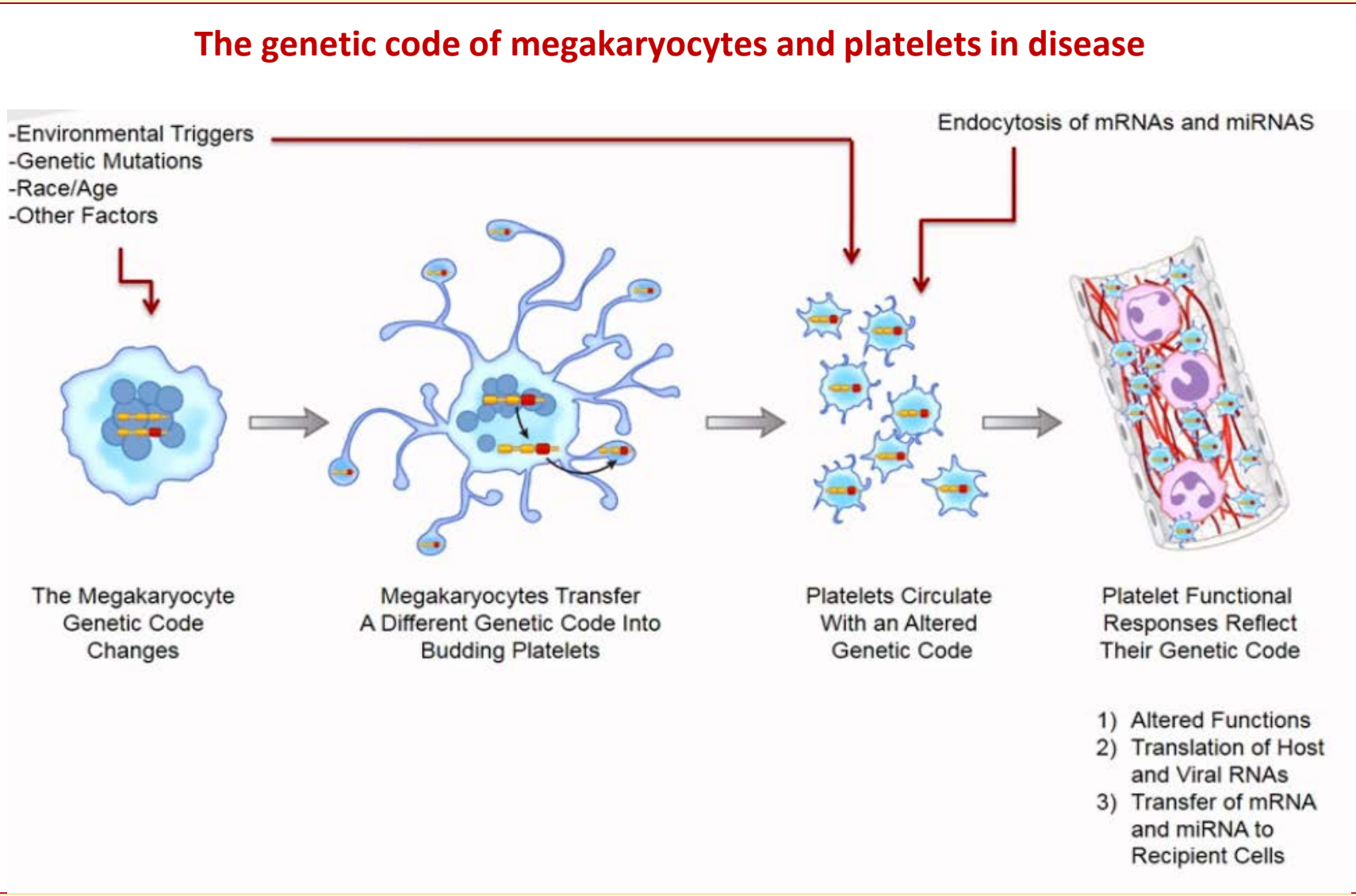
Genetic regulation of megakaryocytes and platelets



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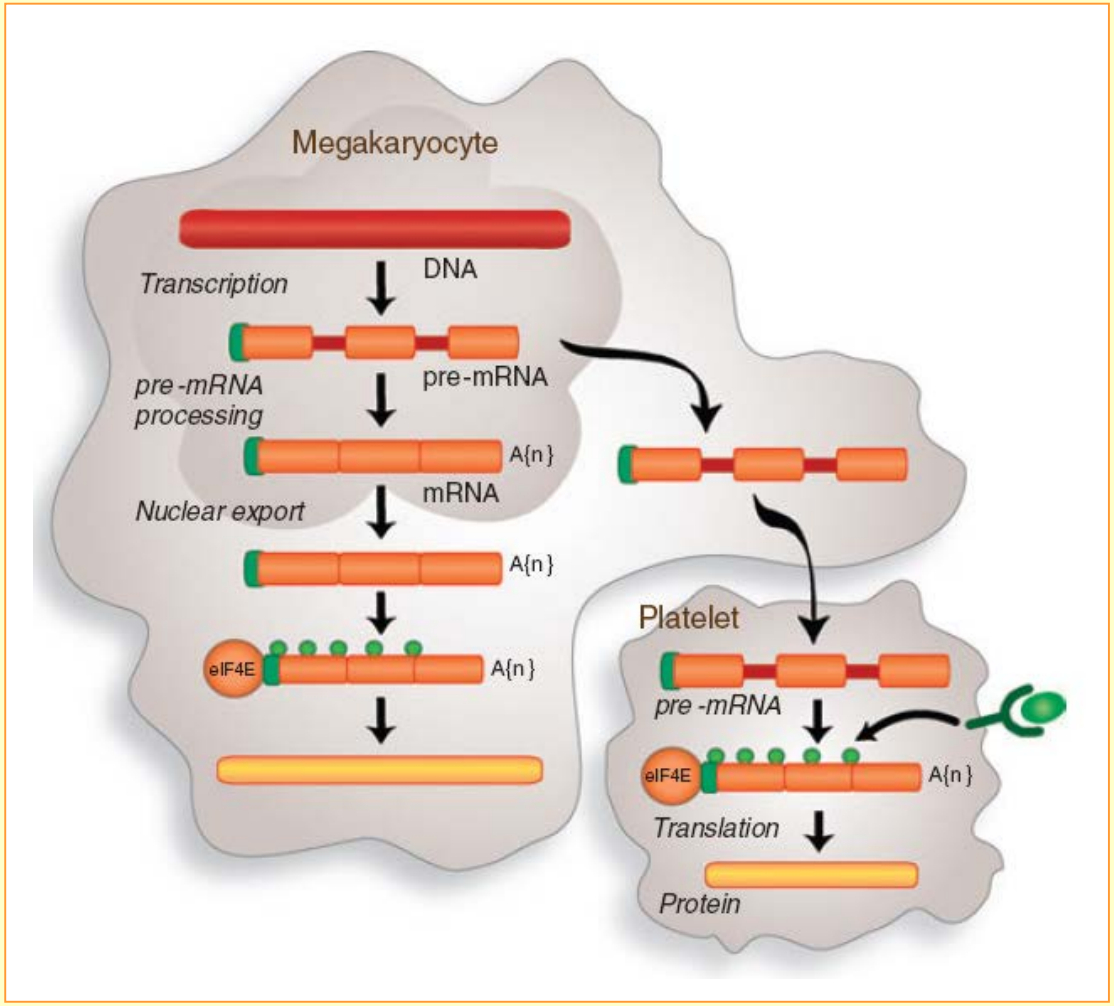
Platelets synthesize new proteins

Activation-dependent pre mRNA-splicing starts protein synthesis

Examples:
- BCL-3
- IL-1β
- COX-1
- COX-2
- PAI-1
- TF
- TIMP-2



Weyrich A et al., J Thromb Haemost 2009, 7:241

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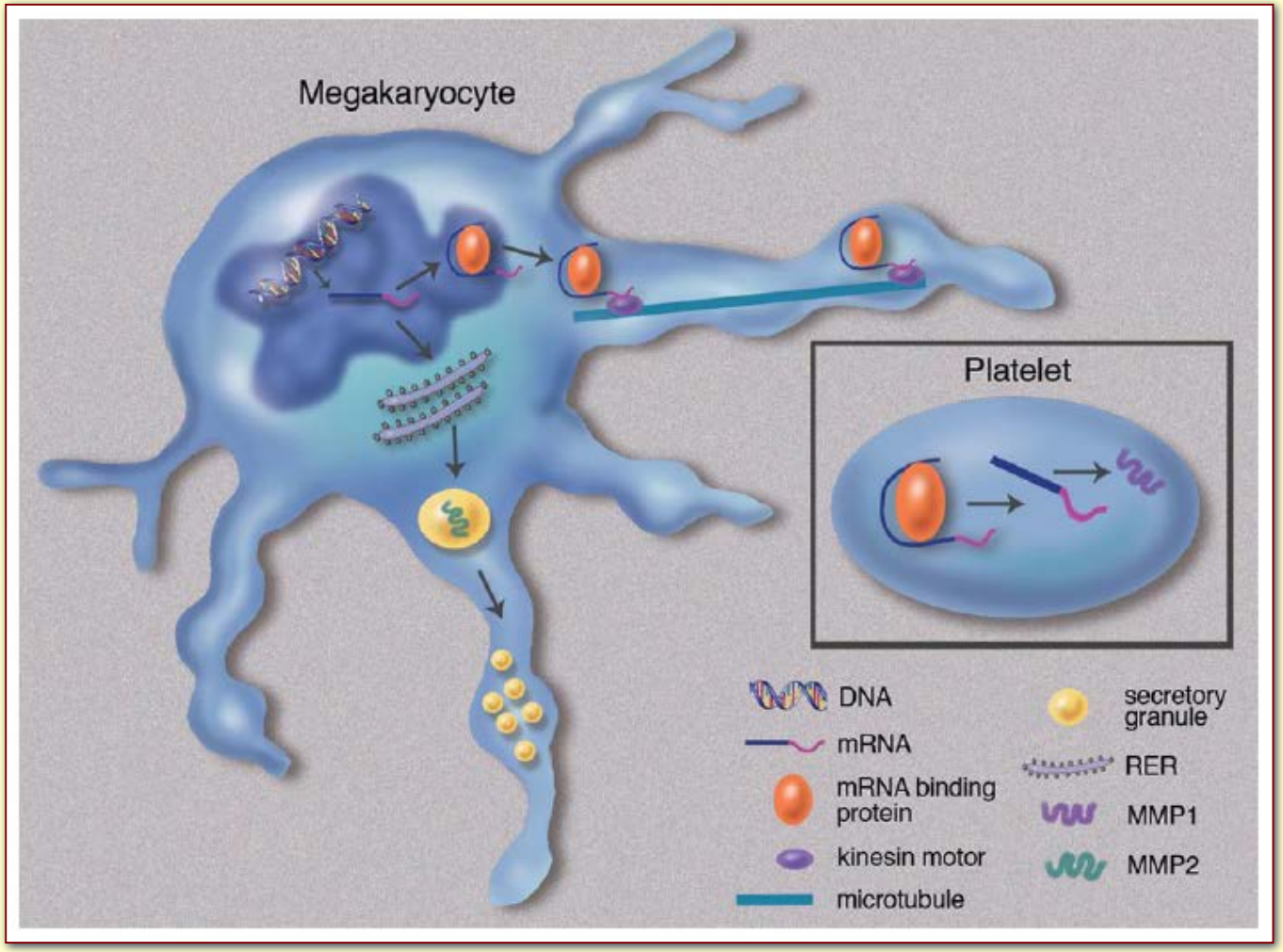
Megakaryocytes differentially sort mRNA for MMPs/TIMPs into platelets



Cecchetti L et al., Blood 2011, 118:1903

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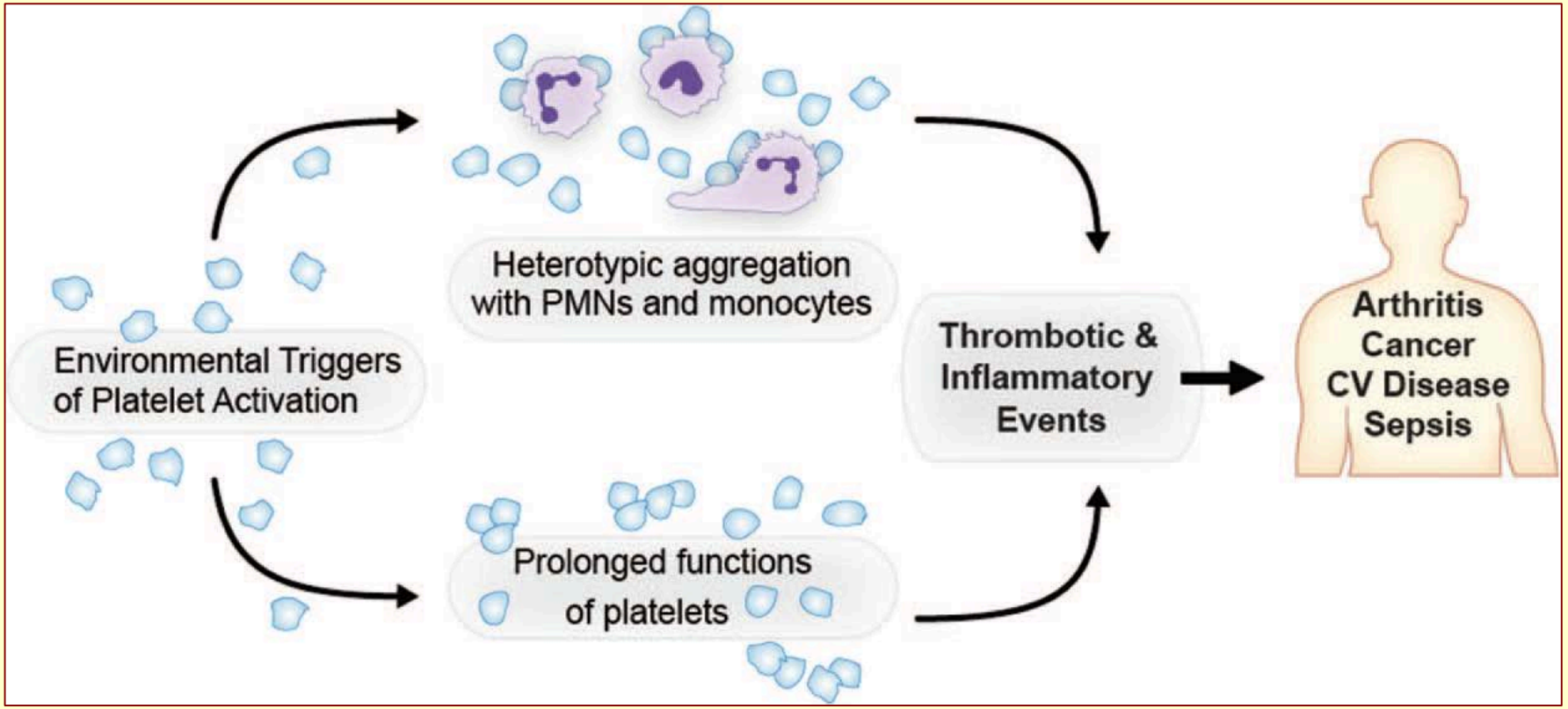
Platelets as effectors of thrombotic and inflammatory injury



Variations in race, genetic mutations, or acute disease are associated with changes in the genetic code (i.e. mRNA and miRNA expression patterns) in megakaryocytes. Changes in the megakaryocyte genetic code are then transferred into developing platelets that are released into the circulation. **Alterations in the platelet transcriptome and corresponding proteins influence functional responses.**

Weyrich AS. Hematology 2014;400

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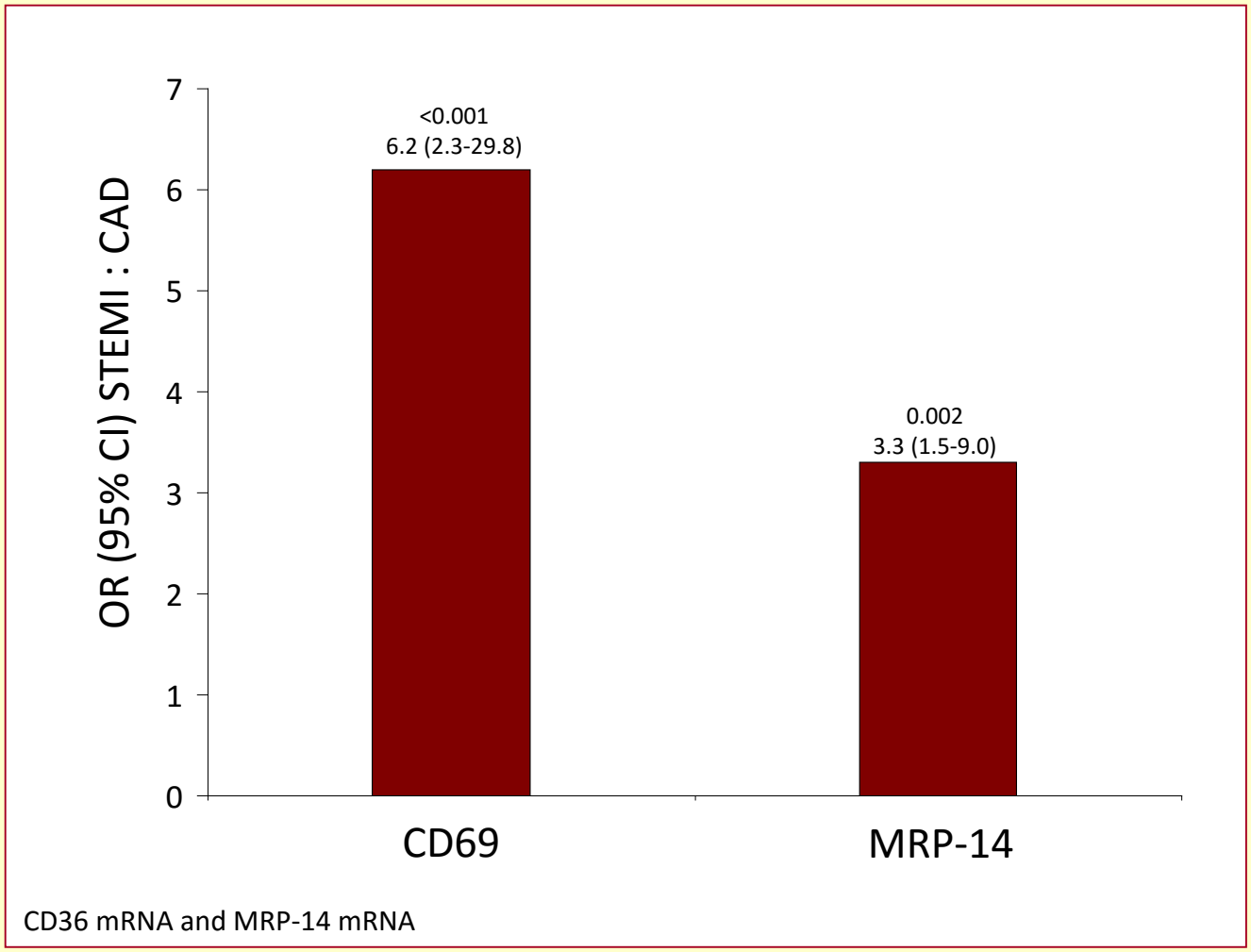
Platelet transcriptome is different between STEMI or CAD patients



CD36 mRNA and MRP-14 mRNA

Healy AM et al., Circulation 2006, 113:2278

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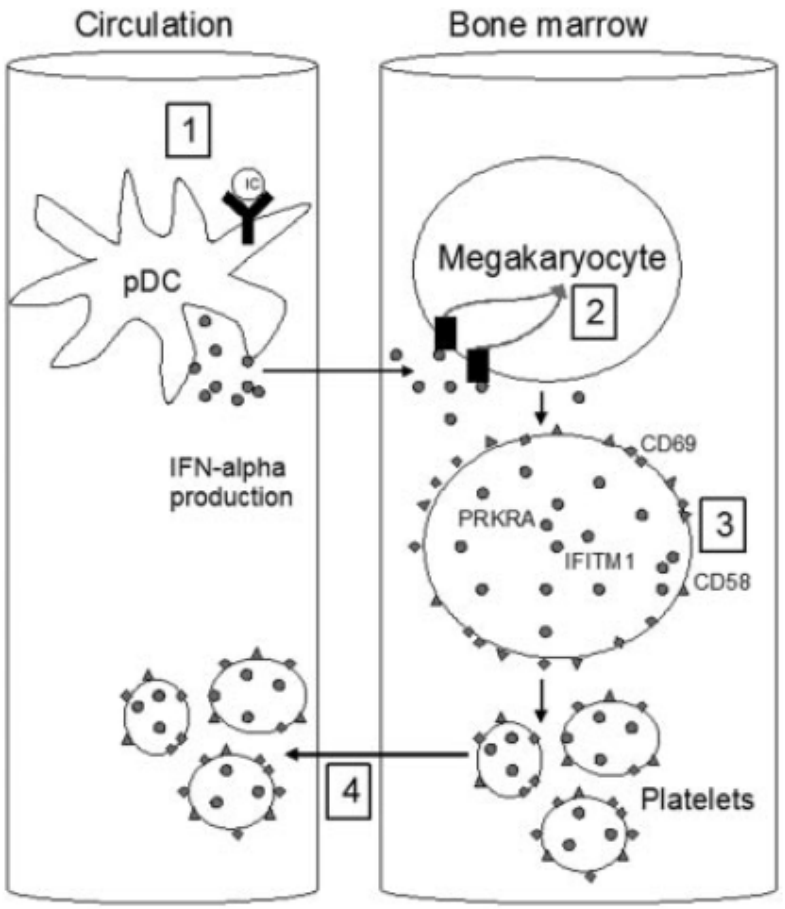
Platelet trascriptome is different between LES patients and healthy controls

Gene symbol	Gene title	Fold change
IFI27	interferon, alpha-inducible protein 27	23.12
*CD58**	CD58 antigen, (lymphocyte function-associated antigen 3)	13.04
*PRKRA**	protein kinase, interferon-inducible double stranded RNA dependent activator	6.34
G1P3	interferon, alpha-inducible protein (clone IFI-6-16)	6.27
*CD69**	CD69 antigen (p60, early T-cell activation antigen)	5.25
*IFITM1**	interferon induced transmembrane protein 1 (9-27)	3.42
OAS1	2′,5′-oligoadenylate synthetase 1, 40/46kDa	2.87
STAT1	signal transducer and activator of transcription 1, 91kDa	2.58
LY6E	lymphocyte antigen 6 complex, locus E	2.43
IFNGR1	interferon gamma receptor 1	2.18
IFRD1	interferon-related developmental regulator 1	2.10
IFI44L	interferon-induced protein 44-like	2.04
OAS3	2′-5′-oligoadenylate synthetase 3, 100kDa	1.98
G1P2	interferon, alpha-inducible protein (clone IFI-15K)	1.98
PLSCR1	phospholipid scramblase 1	1.97
OAS2	2′-5′-oligoadenylate synthetase 2, 69/71kDa	1.92
IRF2BP2	interferon regulatory factor 2 binding protein 2	1.92
GBP1	guanylate binding protein 1, interferon-inducible, 67kDa	1.87
IFI16	interferon, gamma-inducible protein 16	1.83
IFITM3	interferon induced transmembrane protein 3 (1-8U)	1.80
IRF2	interferon regulatory factor 2	1.70



Lood C et al., Blood 2010, 116: 1951-1957

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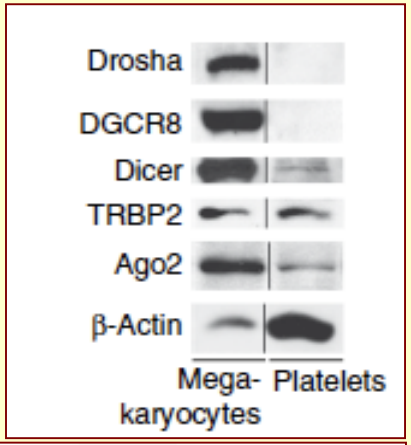
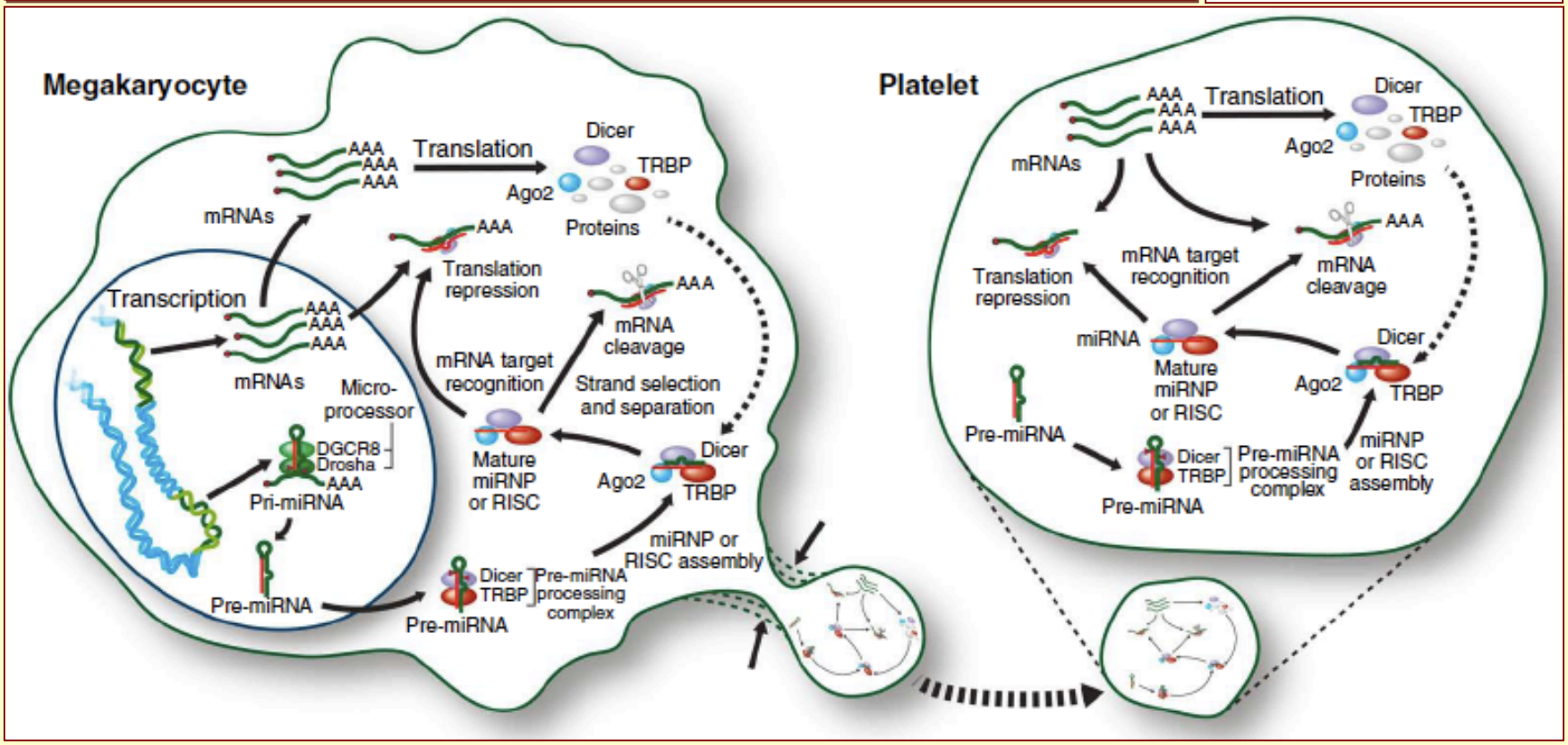
Platelets and miRNA

- Platelets contains miRNAs and the RNAi machinery
- miRNAs regulates gene expression in platelets
- miRNAs have a role in platelet activation





Landry P et al. Nat Struct Mol Biol 2009; 16:961

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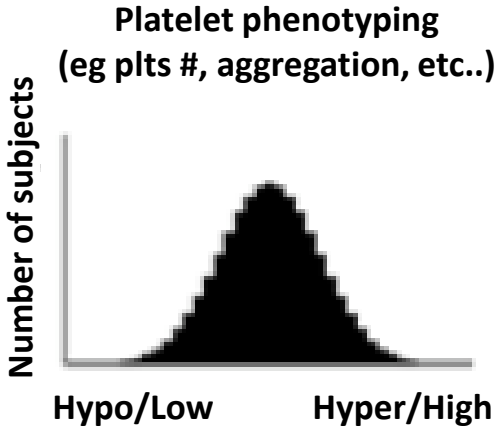
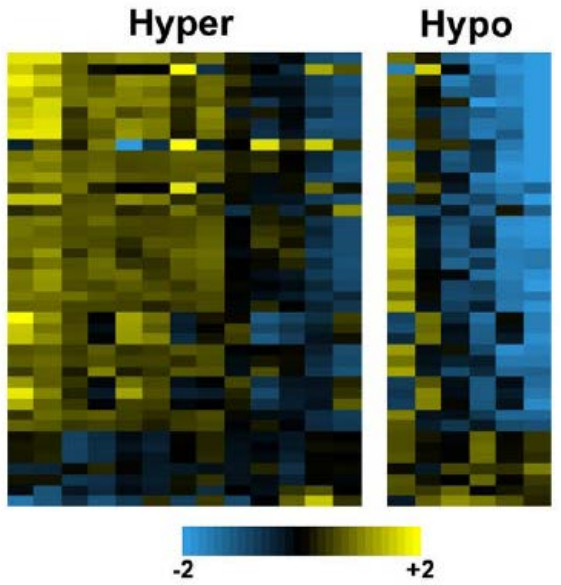
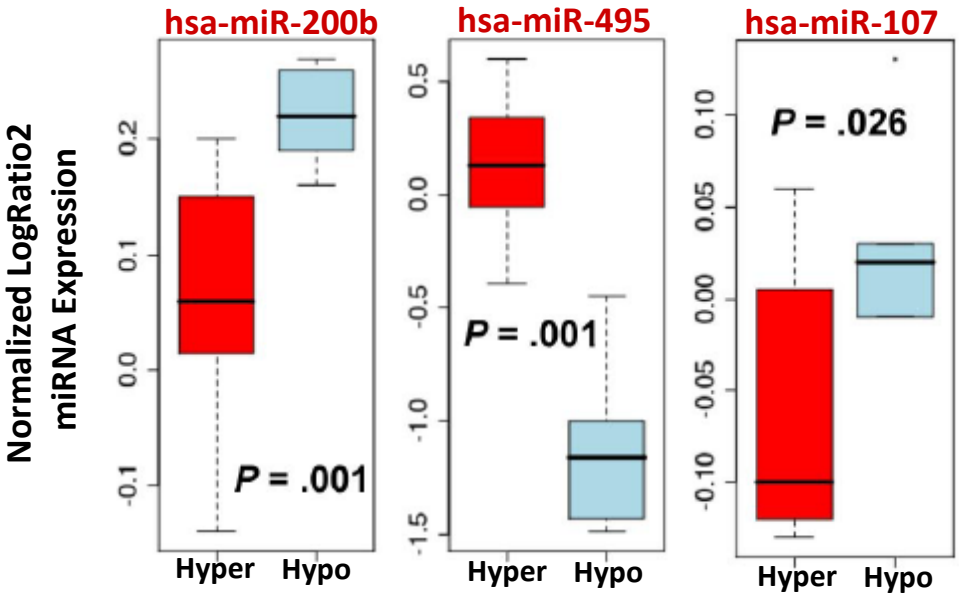
Platelet miRNAs as biomarkers of platelet reactivity







miRNA profiles resulted in 2 groups of subjects that appeared to cluster by platelet aggregation phenotypes.

Nagalla S et al. Blood 2011, 117:5189

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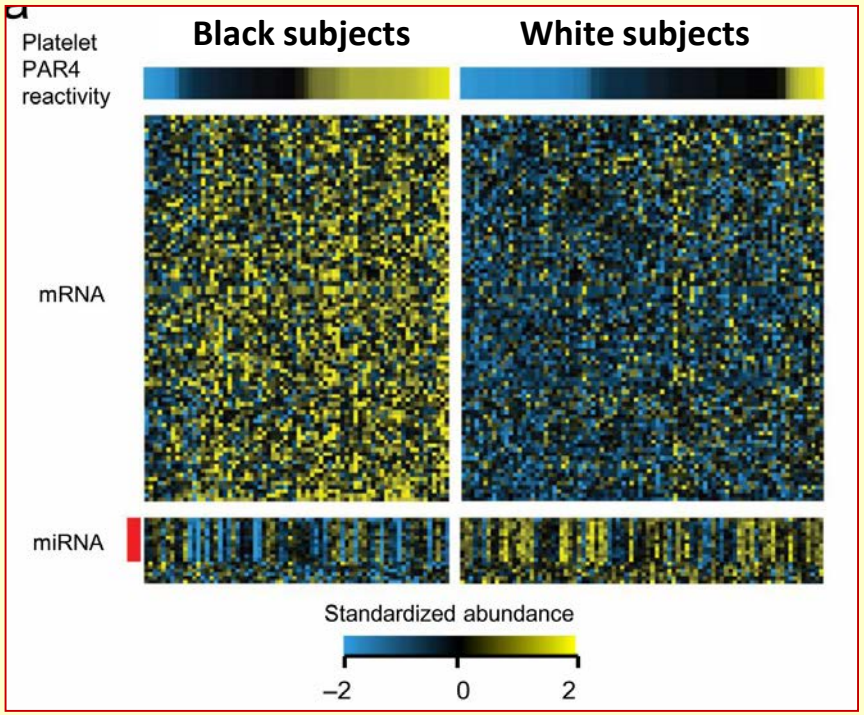
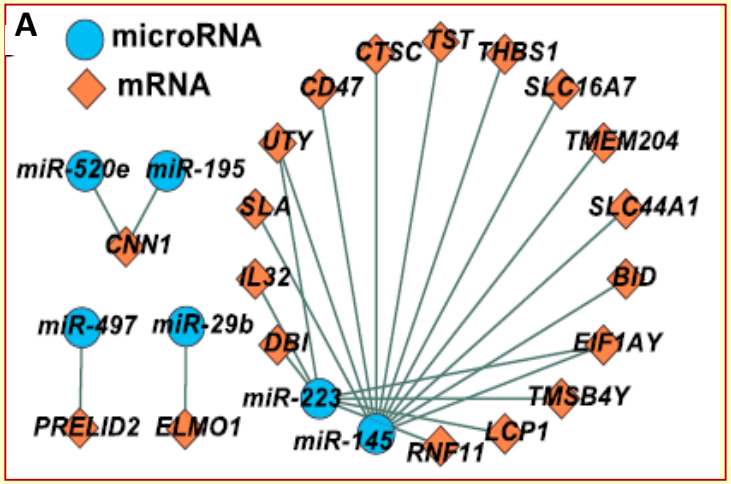
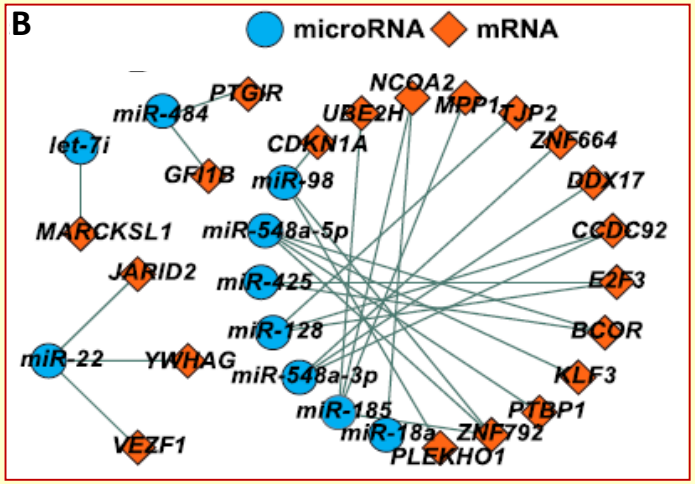
Platelet miRNA-mRNA profiles by race, age and gender



- PAR4 response is higher in blacks
- mRNAs positively correlate with PAR4 response
- miRNAs negatively associate with PAR4 reactivity





Networks of miRNA and mRNA differentially expressed by age (A) and gender (B).

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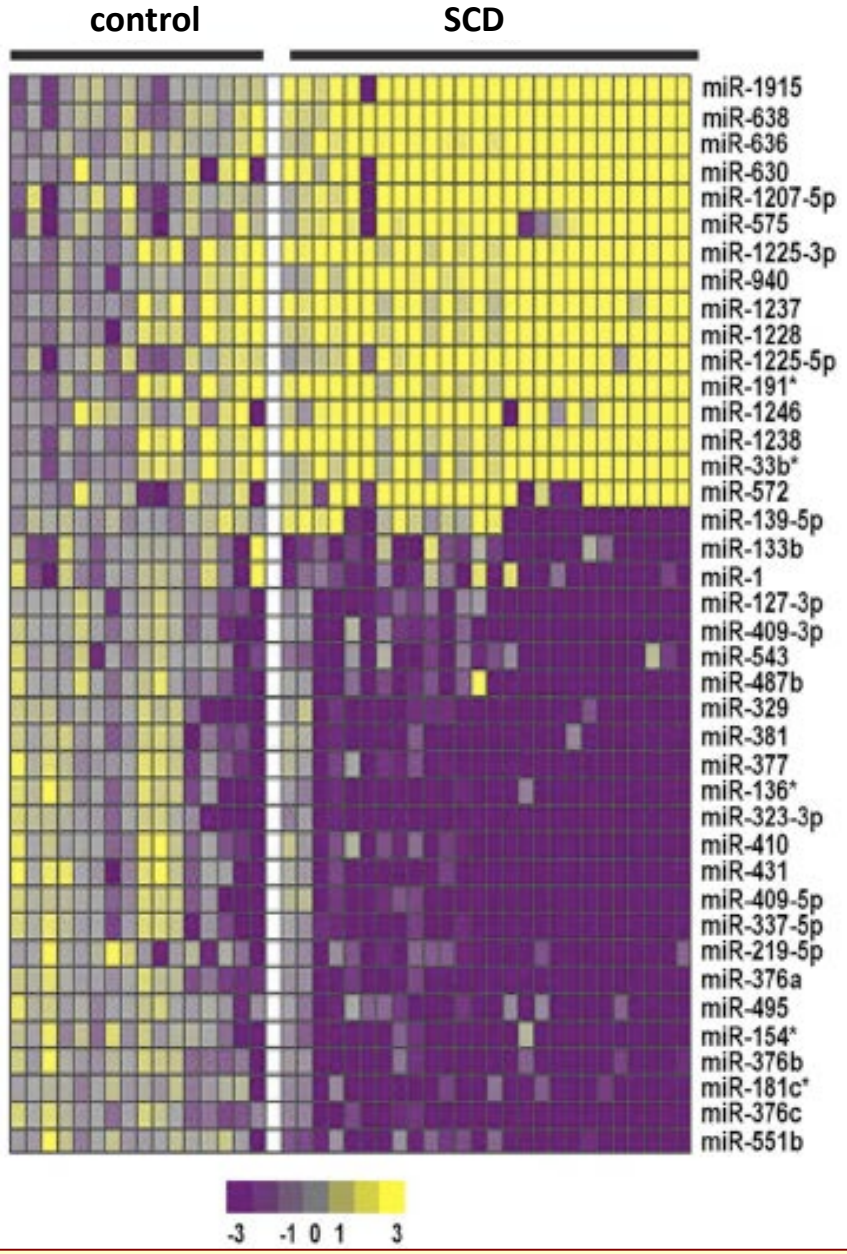
Edelstein LC et al Nat Med. 2013;19(12):1609-16.

Platelet miRNOme in disease



Platelets from patients with sickle cell disease (SCD) exhibit an altered miRNA expression profile.

Up-regulated miRNAs	Down-regulated miRNAs
miR-638	miR-376a
miR-940	miR-381
miR-636	miR-409-3p
miR-1238	miR-377
miR-1225-3p	miR-376c
miR-1207-5p	miR-495
	miR-376b
miR-33b	miR-181c
miR-630	miR-487b
miR-191	miR-337-5p

Jain S et al. PLoSOne 2013, 8:e60932.

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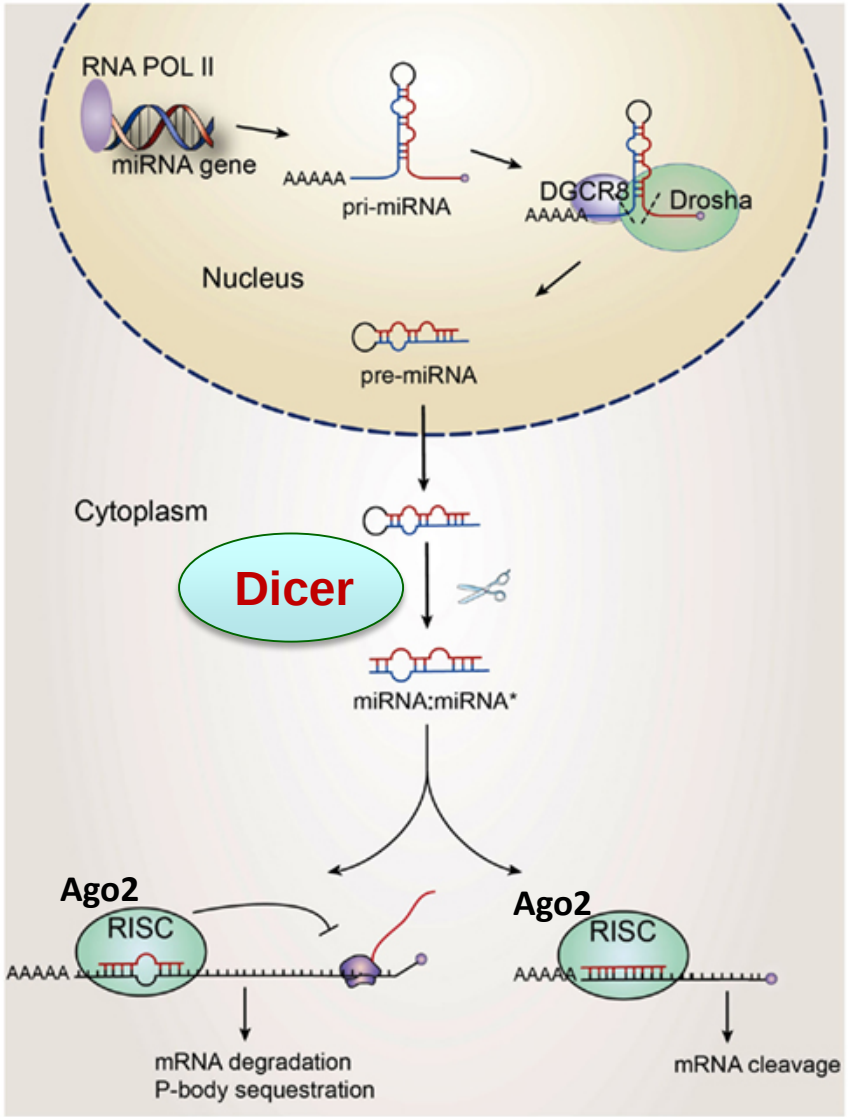
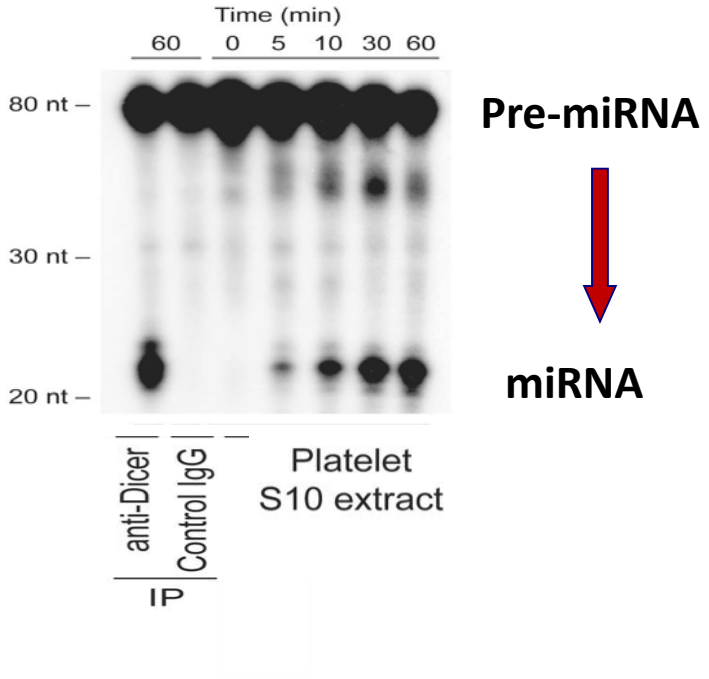
Dicer, the key enzyme of RNA interference, is regulated by thrombin stimulation in human platelets

Giorgia Manni*, Loredana Bury, Alessandro Marturano, Elisa Piselli and Paolo Gresele





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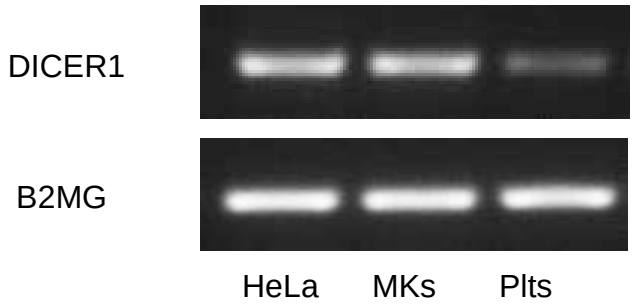
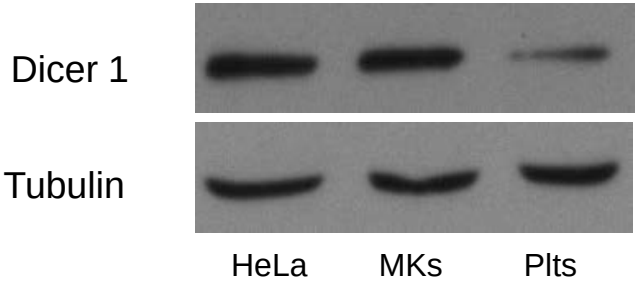
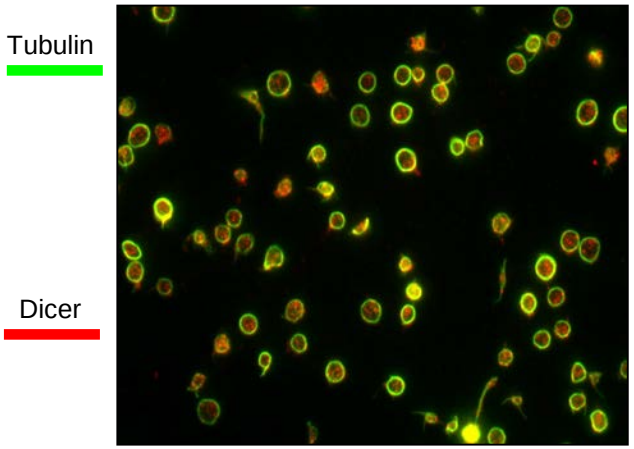
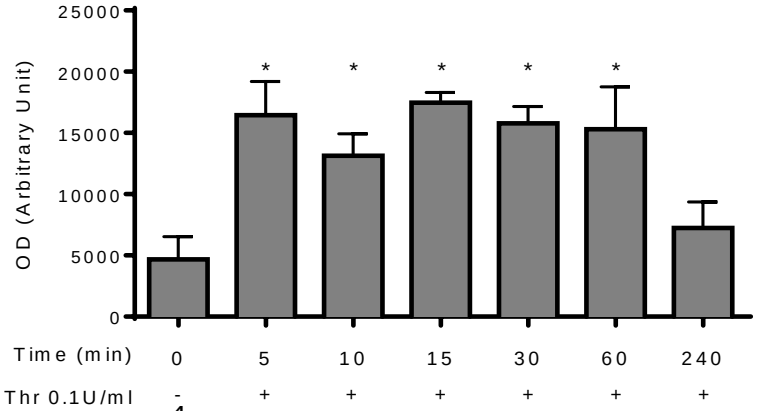
Landry P et al., Nat Struct Mol Biol 2009

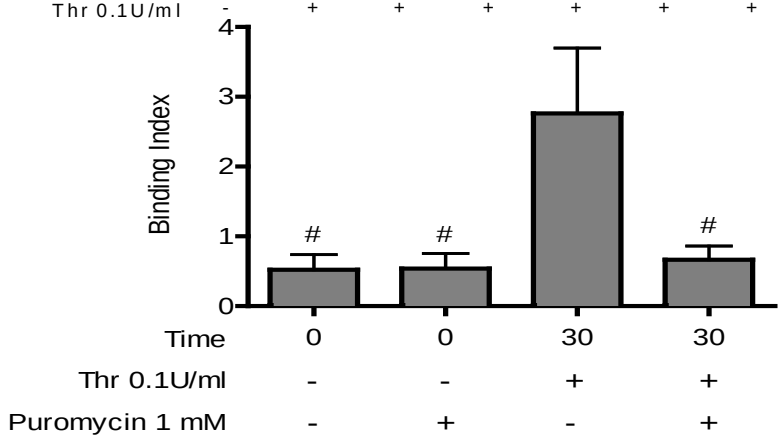
Dicer protein and mRNA are present in resting platelets
Thrombin induces Dicer synthesis

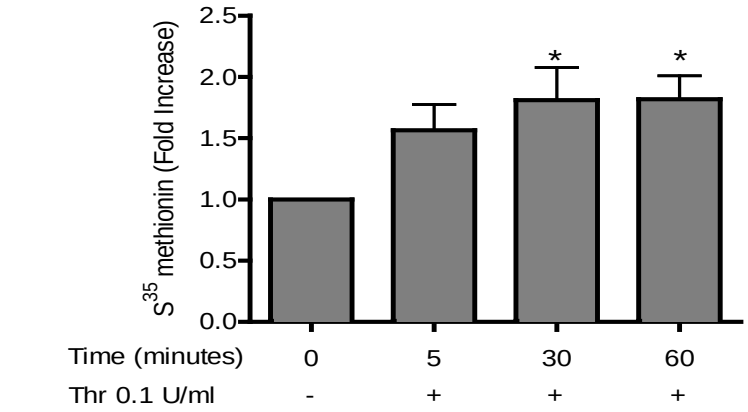




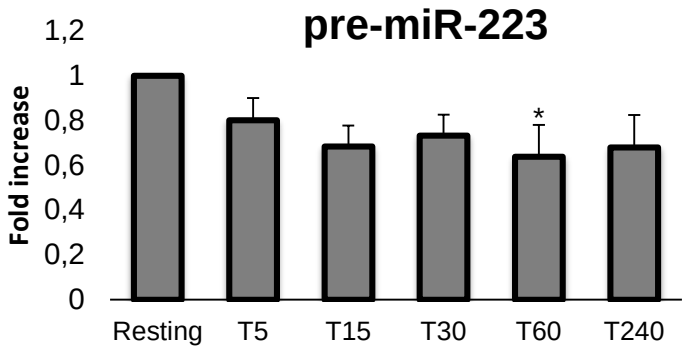


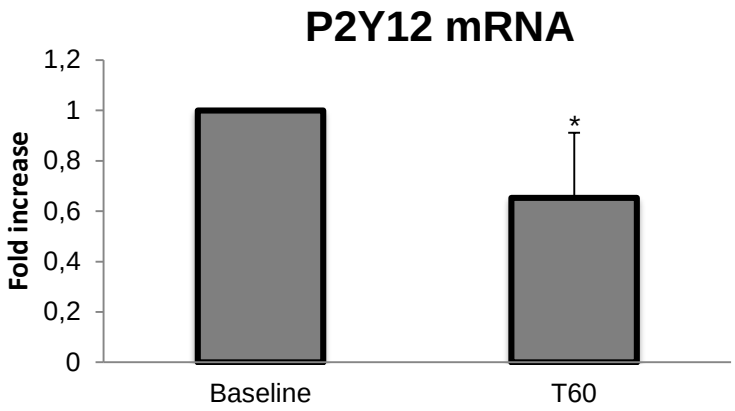


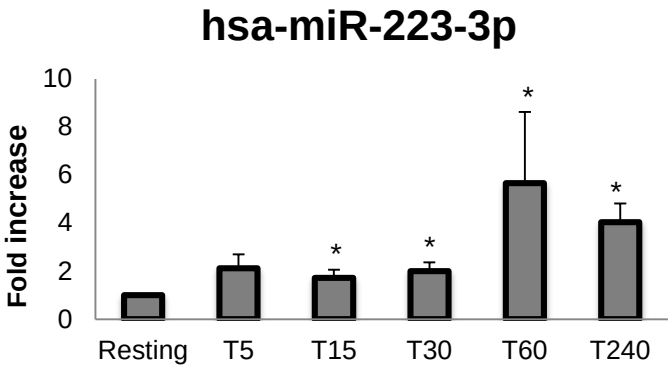


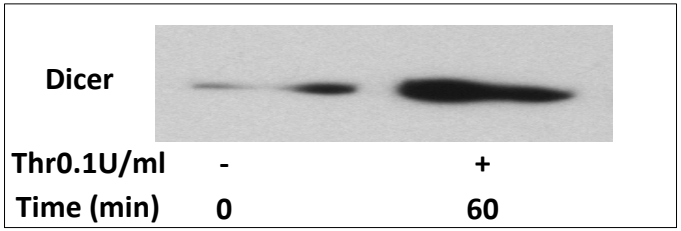


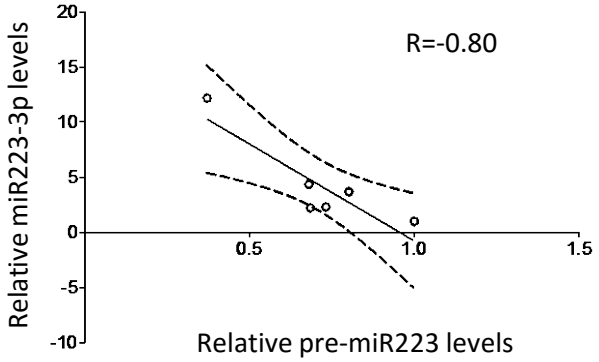
Dicer synthesis is associated with mir-223 maturation and target mRNA down-regulation

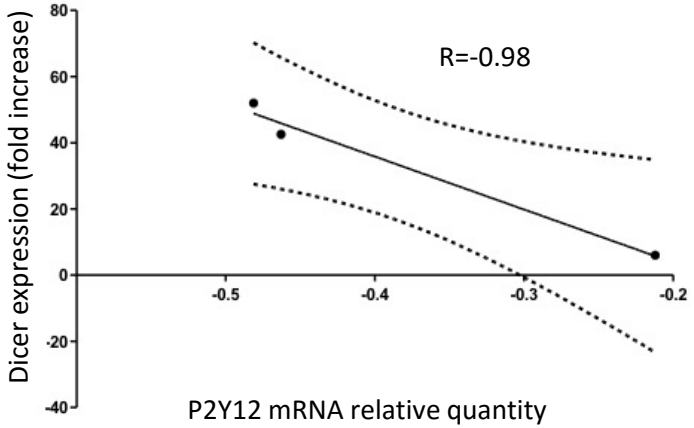






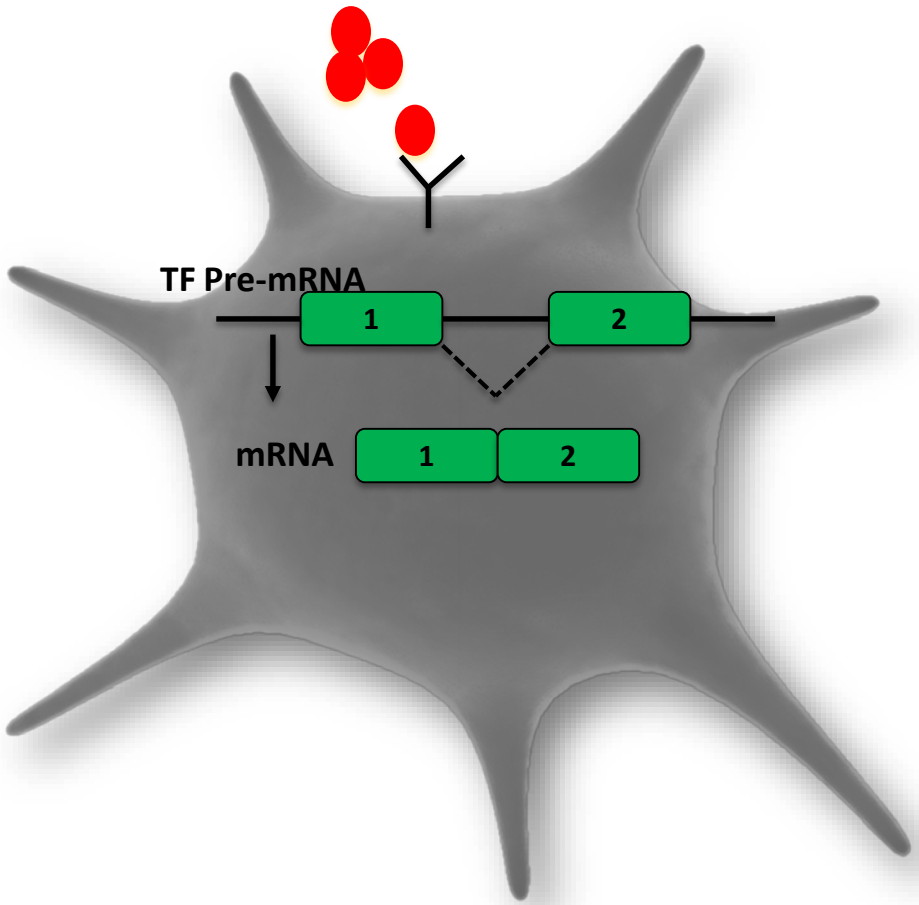






Diphosphorylated lipid A triggers Tissue factor mRNA splicing in human platelets

Krauel K*, Thorack U, Schumacher A, and Schwertz H

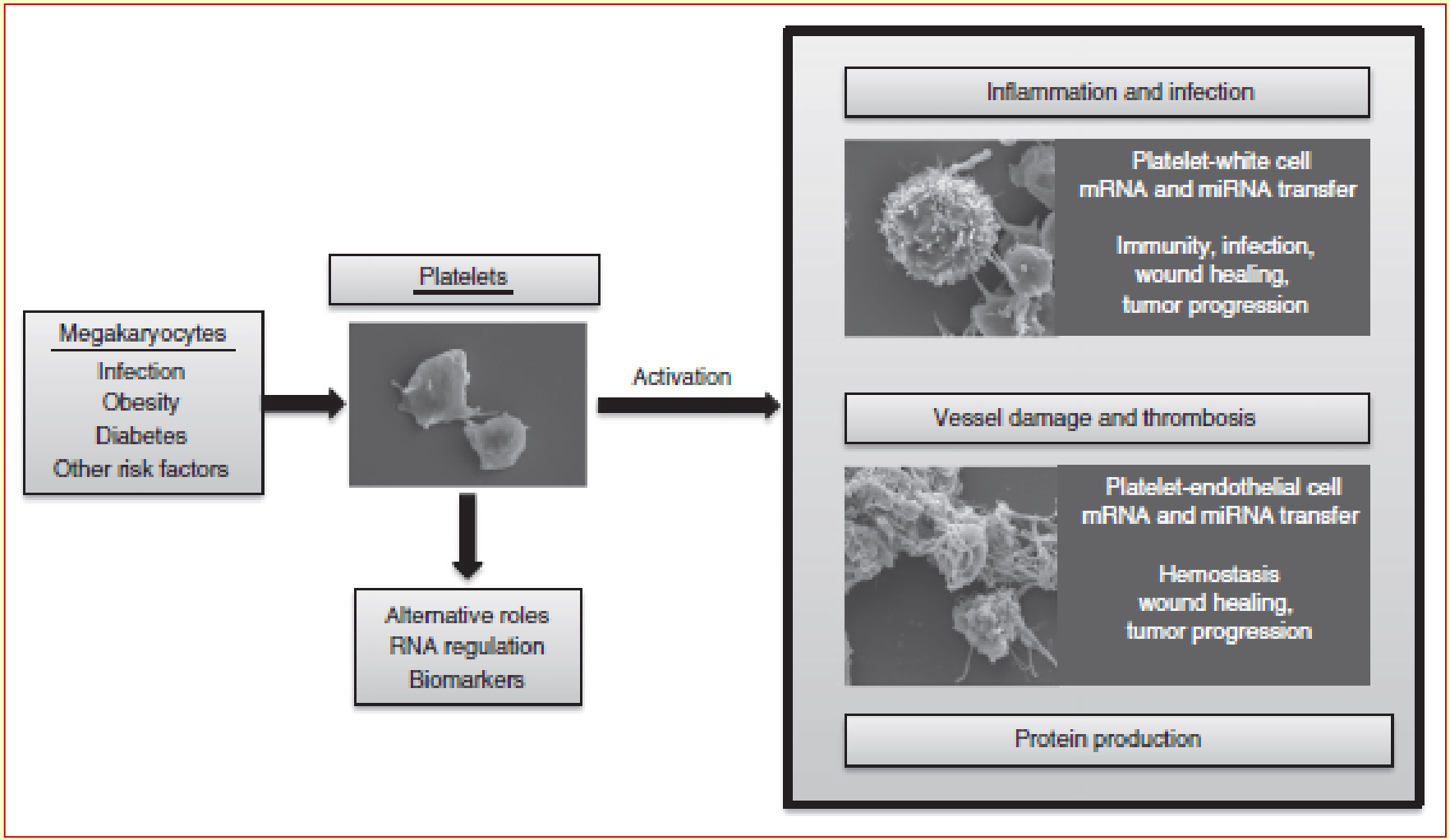


- LPS is an agonist for platelet mRNA splicing of TF.
- LPS components are lipid A di- and mono-phosphate and 3-deoxy-D-manno-2-octulosonic acid (KDO).
- LPS and lipid A diphosphate, but not monophosphate or KDO, trigger mRNA splicing.
- TF mRNA required LPS activation for maturation, while IL-1β mRNA was already mature at baseline
- Lipid A diphosphorylated is required to trigger TF mRNA-splicing in human platelets

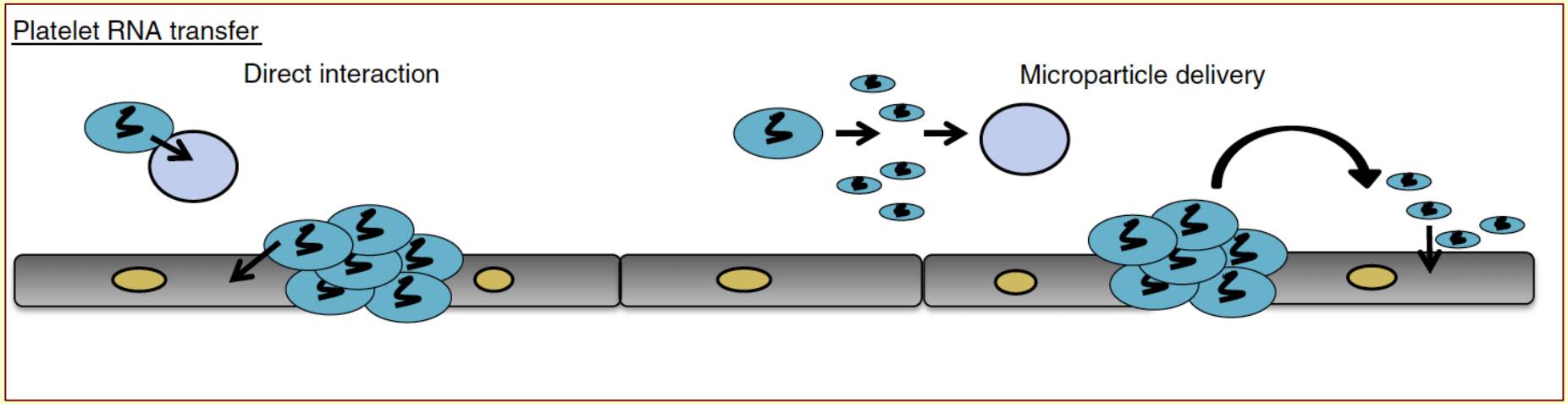
The role of circulating platelet transcripts

Jane E. Freedman

State of the art lecture



Platelet RNA transfer mechanisms



Platelet RNA transfer may affect vascular cell expression and homeostasis by:

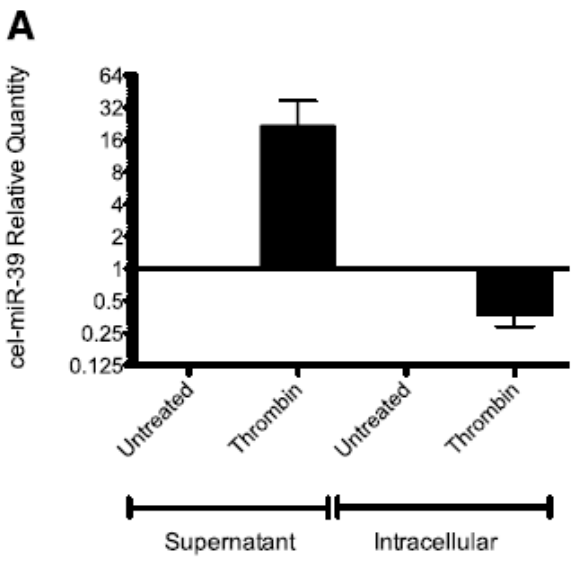
- direct interaction of platelets with white blood and/or endothelial cells
- microparticles formation and subsequent interaction of microparticles with white blood and/or endothelial cells

Clancy L, Freedman JE. J Thromb Haemost 2015;13: S33–S9.

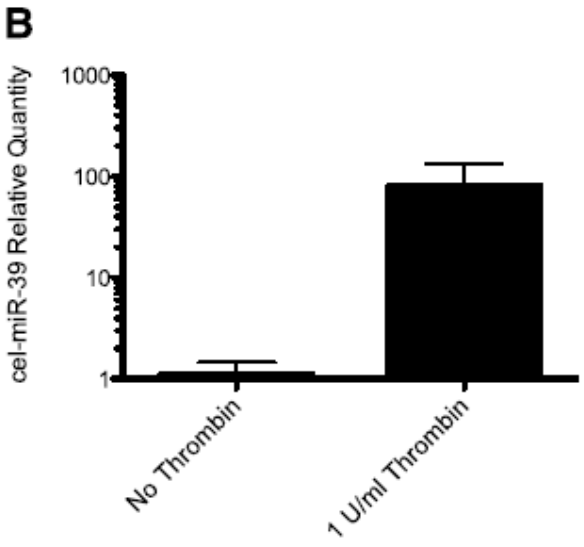
Platelet RNA transfer mechanisms

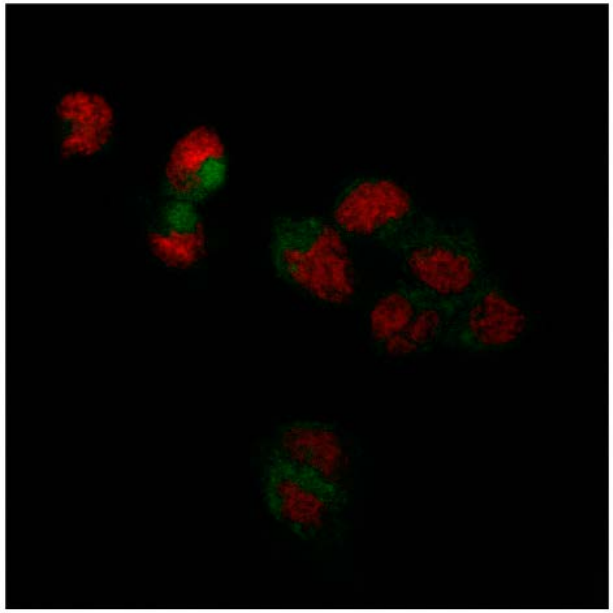
Activated platelets transfer miRNA to endothelial cells in vitro

miR-39 in resting and activated platelets



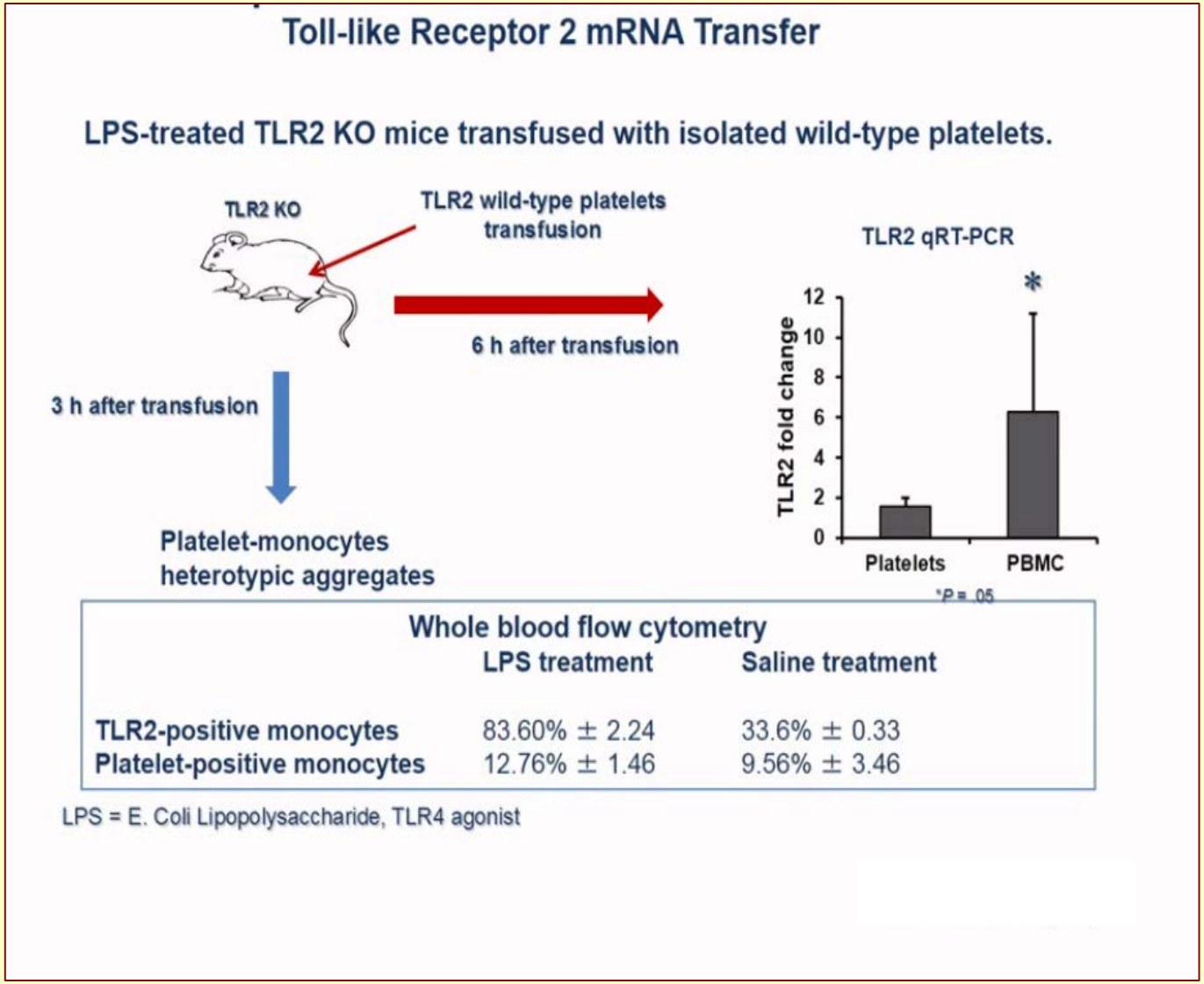
miR-39 in endothelial cells cocultured with miR-39 transfected platelets





Gidlof O et al., Blood 2013;121:3908

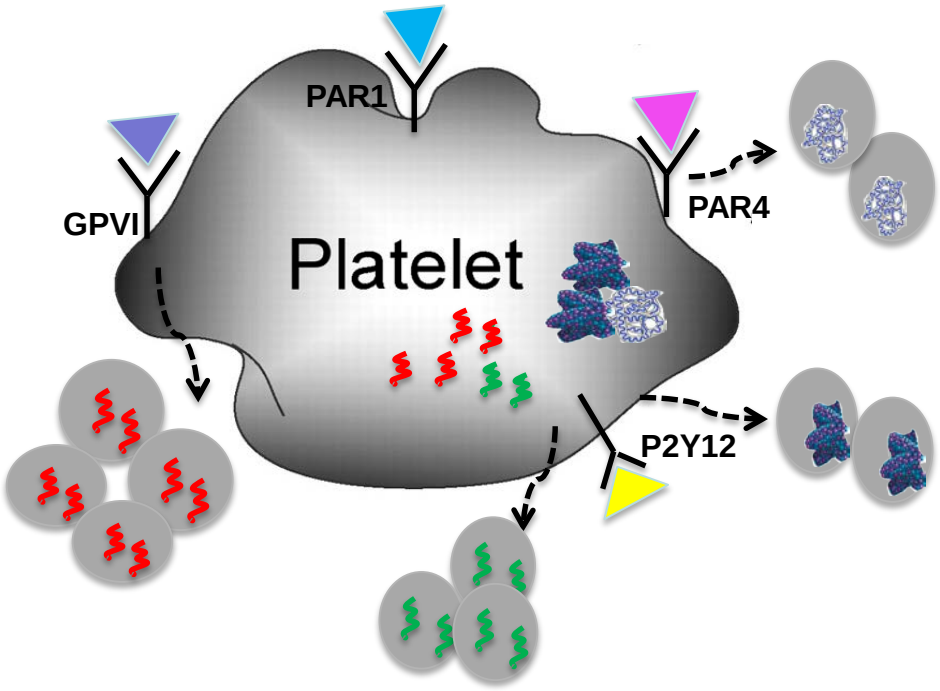
Platelet mRNA transfer occurs *in vivo*



Risitano A et al., Blood 2012;119:6288

Platelets release extracellular vesicles in an agonist dependent manner but release a consistent profile of microRNA

Amrose AR, Pringle JH and Goodall AH

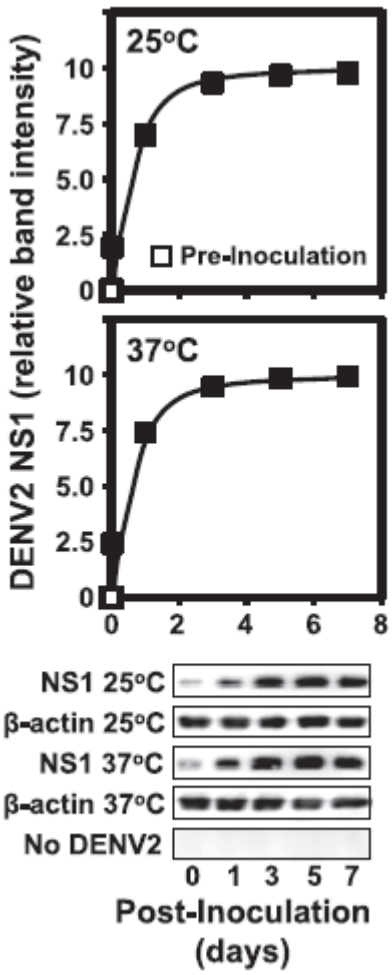


- Platelets contain abundant microRNAs which can be packaged into extracellular vesicles (EV) and released into the circulation.
- EVs contained between 57 and 79 different miRNAs depending on the stimulus.
- miR-223 was expressed in all samples

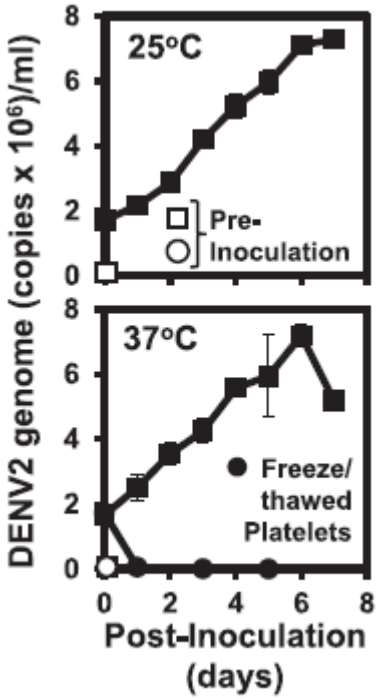
These data suggest that the EVs released from platelets and their miRNA content are agonist-dependent

Dengue virus pirates human platelets

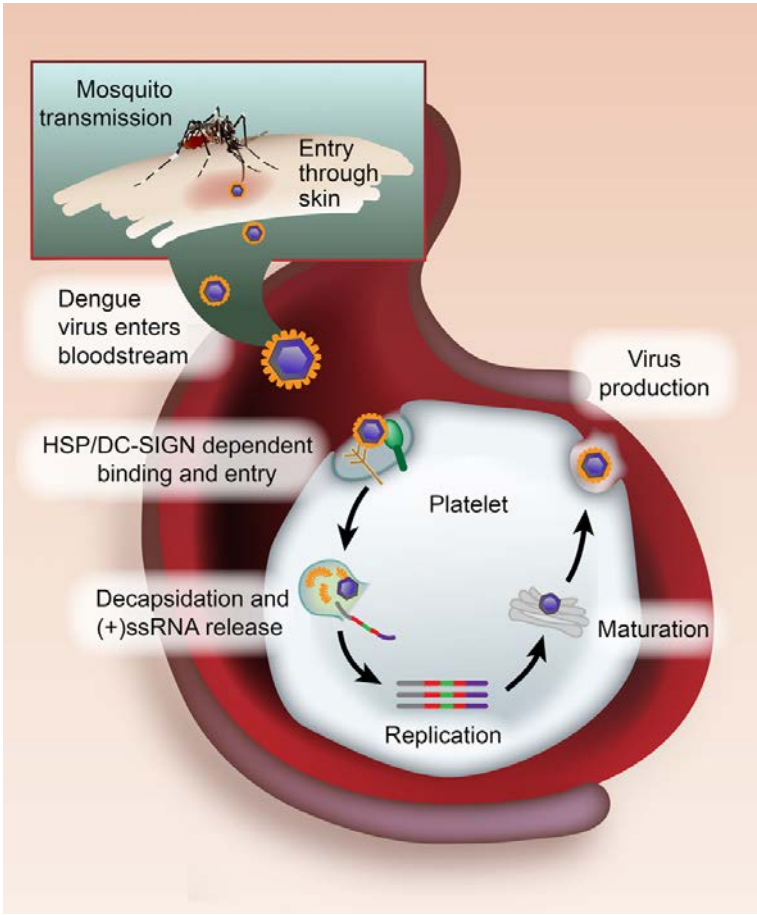
Dengue virus protein is translated by platelets



Platelet-dependent replication of Dengue virus genome



Dengue virus (DENV) raids platelets and steals their translational machinery to replicate and produce infectious virus.

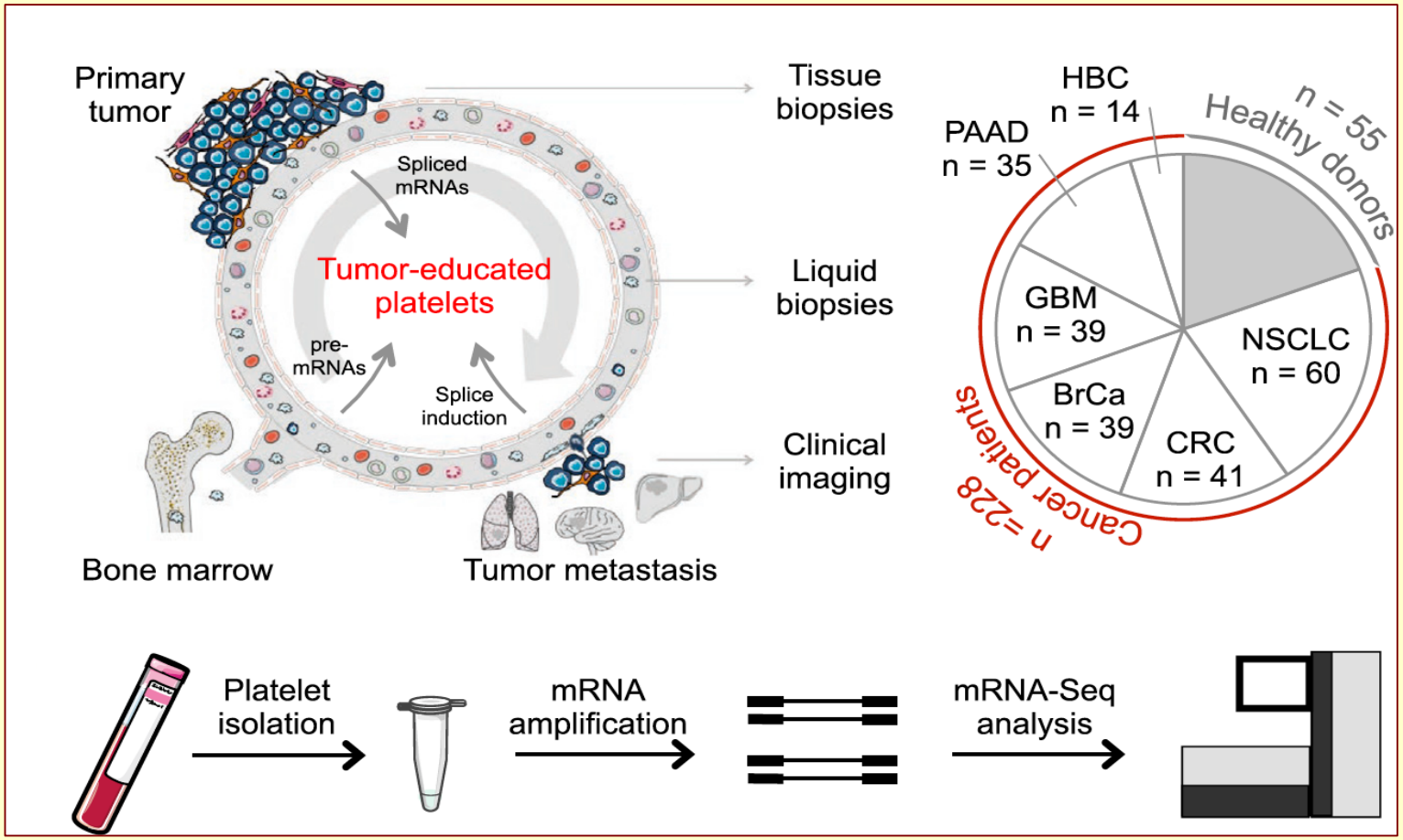


Simon AY et al., Blood 2015;126:378

RNA signatures in platelets from cancer patients

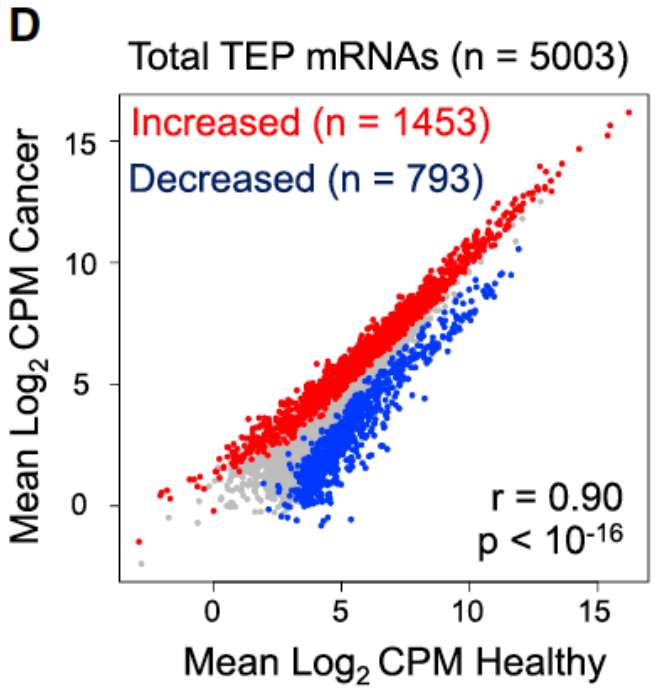
Thomas Wurdinger

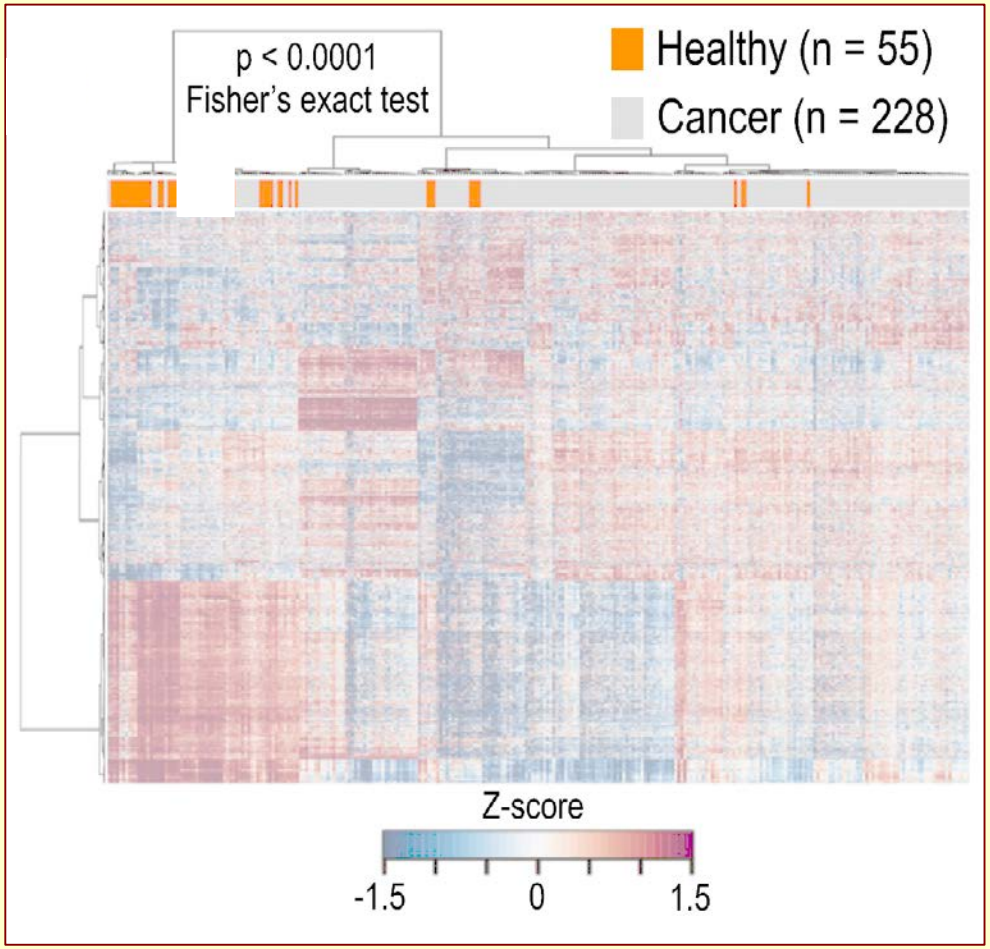
SSC Platelet Physiology Lecture



«…splice events in response to external signals and the capacity of platelets to ingest spliced circulating mRNAs can provide tumor educated platelets with a highly dynamic mRNA repertoire…»

RNA-Seq of Tumor-Educated Platelets (TEPs)

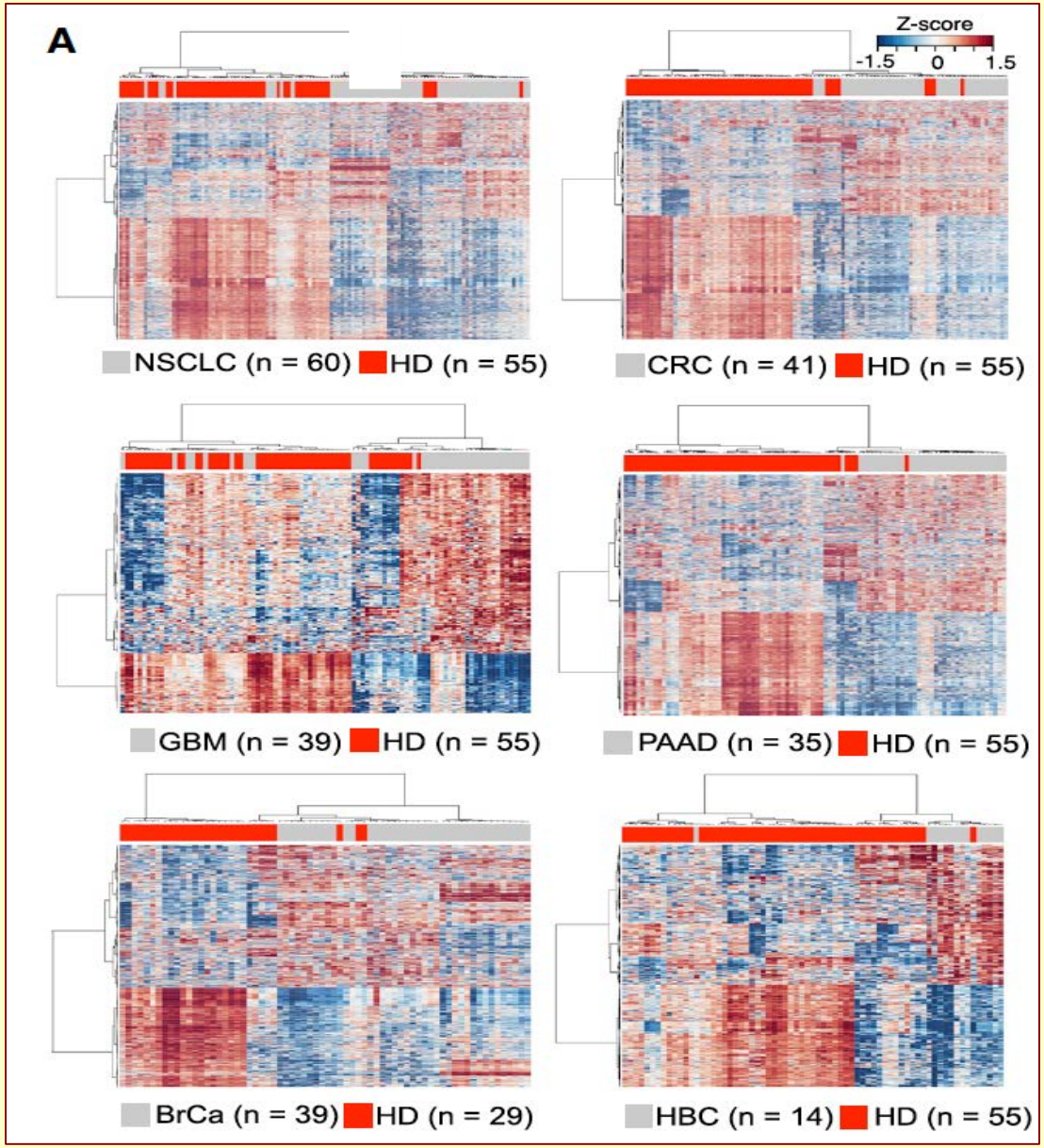




- 5003 different protein-coding and non-coding RNAs were detected in platelets of healthy donors and cancer patients
- 1453 mRNAs were increased and 793 decreased in TEPs
- Differentially-detected mRNAs distinguished two groups with minor overlap

Best MG et al., Cancer Cell 2015, 28:666

RNA-Seq of Tumor-Educated Platelets (TEPs)



In addition to the pan-cancer diagnosis, the TEP mRNA profiles also distinguished healthy donors and patients with specific types of cancer, as demonstrated by the hierarchical clustering of differential platelet mRNA levels of healthy donors and all six individual tumor types.

NSCLC: non small cell lung
CRC: colorectal
GBM: glyoblastoma
PAAD: pancreatic
BrCa: breast
HBC: hepatobiliary

Best MG et al., Cancer Cell 2015, 28:666

RNA-Seq of Tumor-Educated Platelets

TRAINING COHORT

Actual Class

Predicted Class	Cancer	Healthy	Genes: 1072 Total (*n*)
Cancer	131 (96%)	3 (8%)	134
Healthy	5 (4%)	36 (92%)	41
	136 (100%)	39 (100%)	175

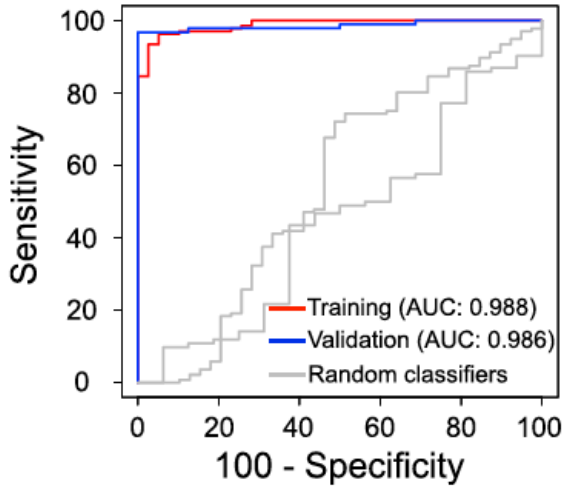
Accuracy: 95%, $p < 0.01$

VALIDATION COHORT

Actual Class

Predicted Class	Cancer	Healthy	Genes: 1072 Total (*n*)
Cancer	89 (97%)	1 (6%)	90
Healthy	3 (3%)	15 (94%)	18
	92 (100%)	16 (100%)	108

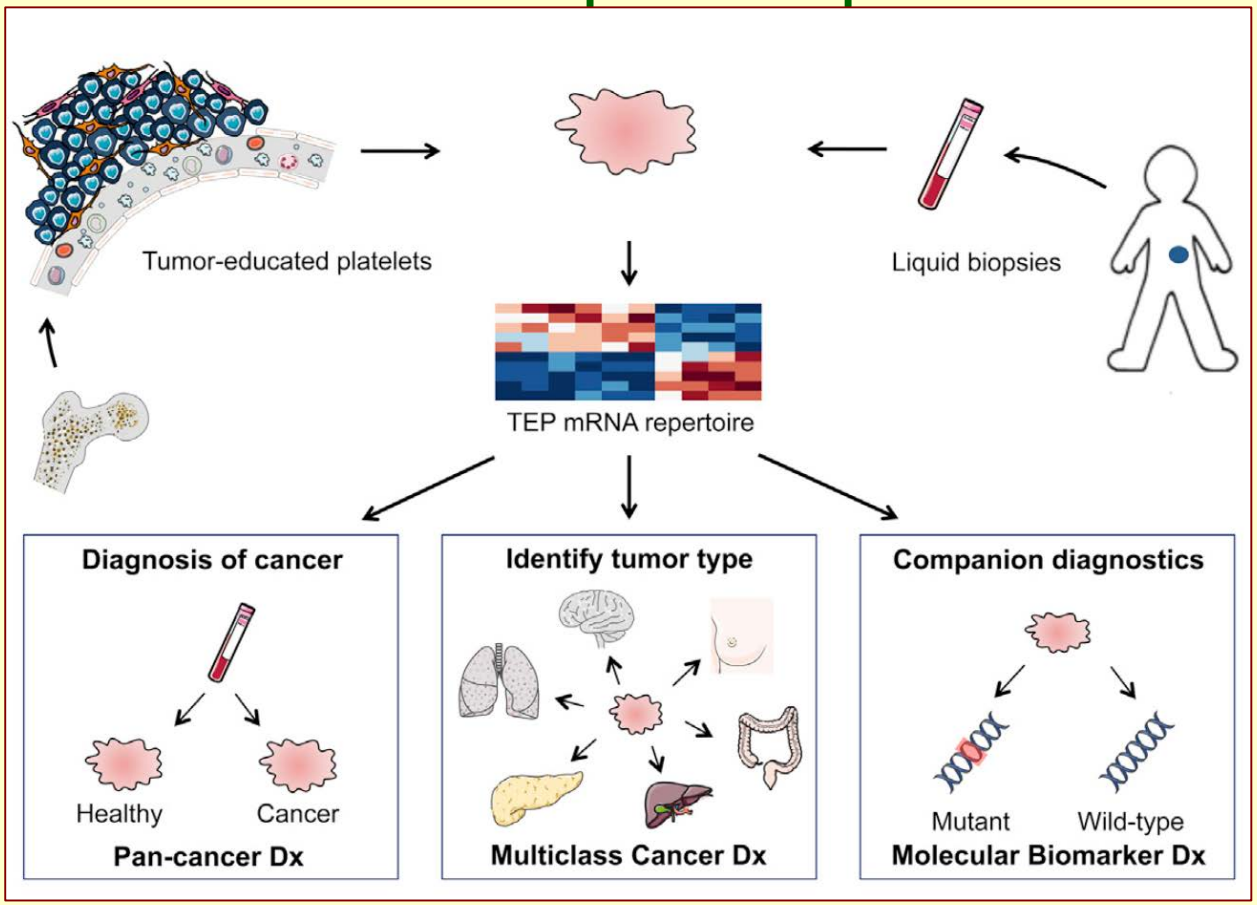
Accuracy: 96%



Pan-cancer TEP-test	Correctly classified	Mean predictive strenght (min-max)
Localized cancer	39/39 (100%)	0.99 (0.94-1.0)
Metastasized cancer	181/189 (96%)	0.96 (0.53-1.0)
Healthy donors	51/55 (93%)	0.87 (0.5-1.0)
Primary CNS tumors	33/39 (85%)	0.89 (0.54-1.0)
Adenocarcinomas	188/190 (99%)	0.98 (0.71-1.0)

Best MG et al., Cancer Cell 2015, 28:666

Blood platelets represent an advancement in blood-based “liquid biopsies”



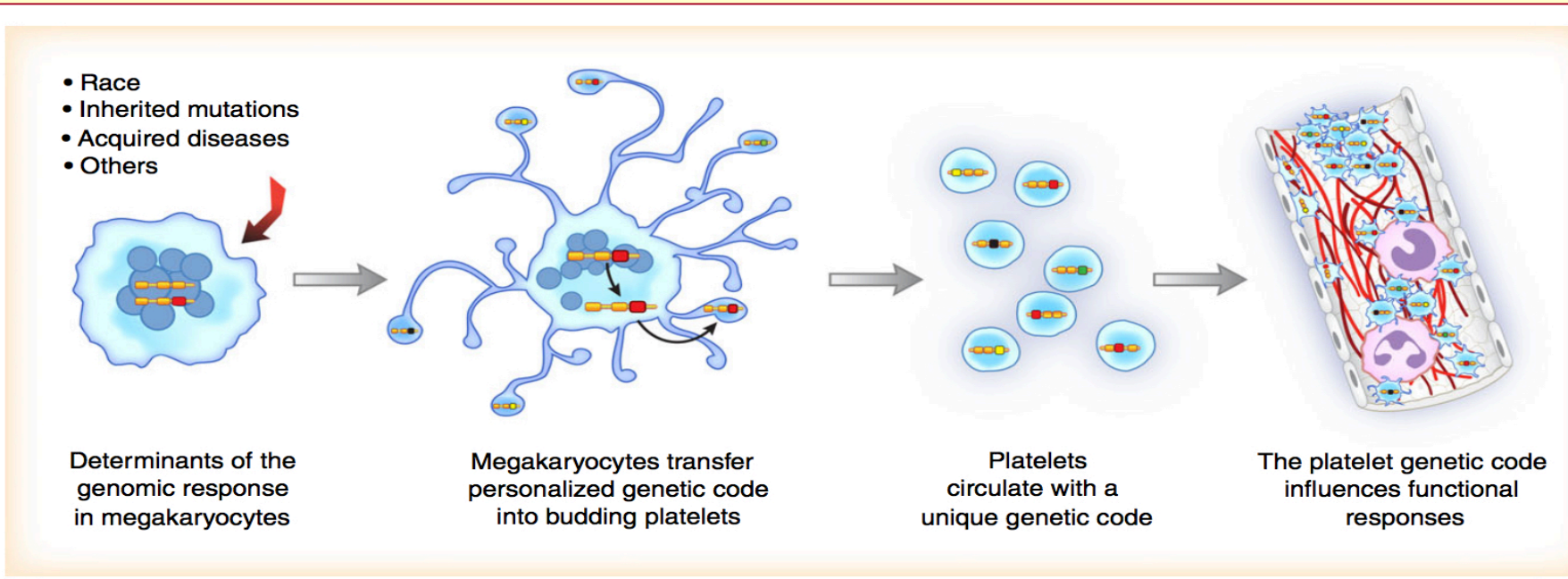
- Tumor educated platelets (TEPs) provide a RNA biosource for pan-cancer, multiclass, and companion diagnostics
- Blood platelets are a potential all-in-one platform for blood-based cancer diagnostics, using the equivalent of one drop of blood.

Best MG et al., Cancer Cell 2015, 28:666

CONCLUSIONS

- The crucial role of platelets in hemostasis, inflammation, host defence has led to the evolutionary development of finely tuned mechanisms regulating platelet phenotype
- Alterations of platelet and megakaryocyte transcriptome/miRNome play a role in their participation in disease
- The expanding knowledge of these mechanisms may in perspective lead to the identification of novel therapeutic targets and/or to the use of platelets as therapeutic carriers

paolo.gresele@unipg.it

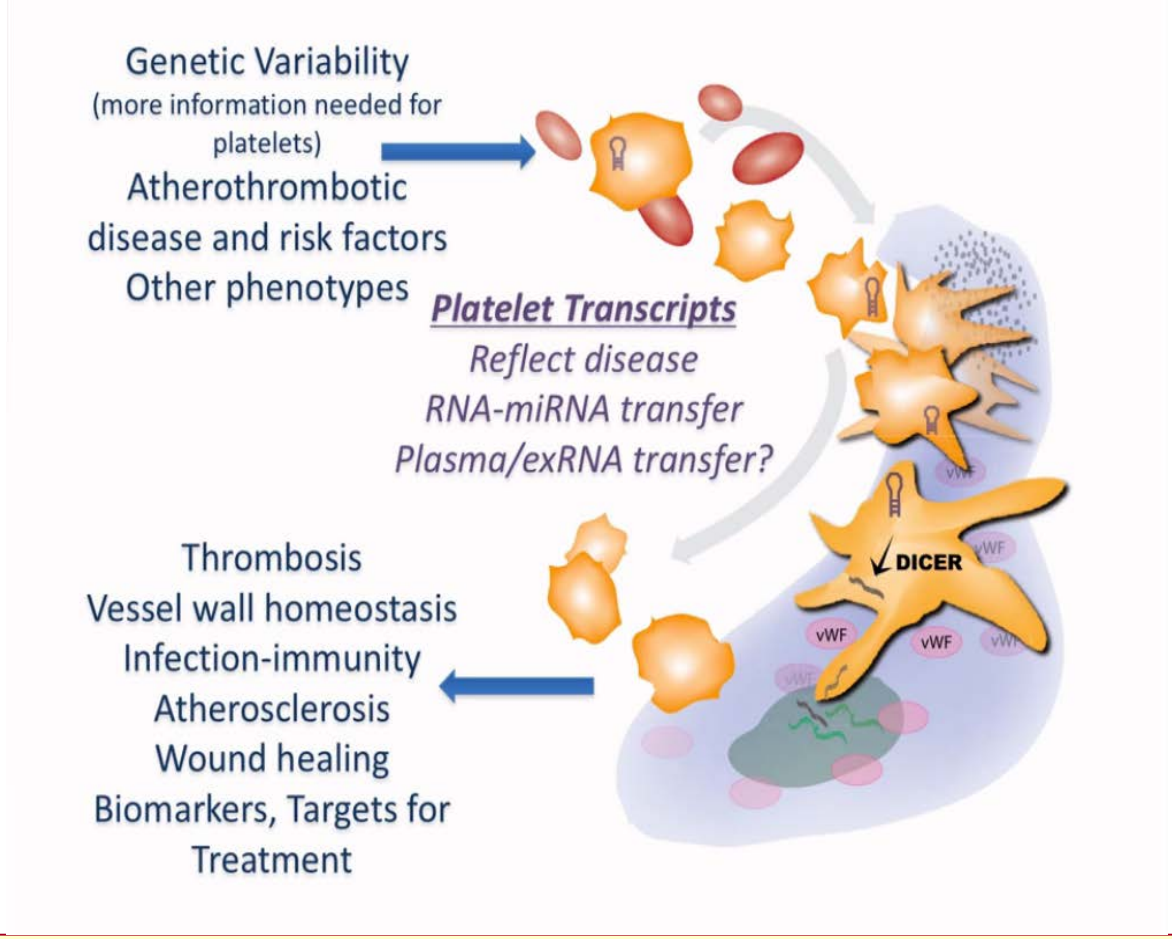

• Race
• Inherited mutations
• Acquired diseases
• Others
Determinants of the genomic response in megakaryocytes
Megakaryocytes transfer personalized genetic code into budding platelets
Platelets circulate with a unique genetic code
The platelet genetic code influences functional responses

Jane E. Freedman

The role of circulating platelet transcripts

State of the art

Platelet Transcriptome: Does it have a direct effect?



Andrew S. Weyrich

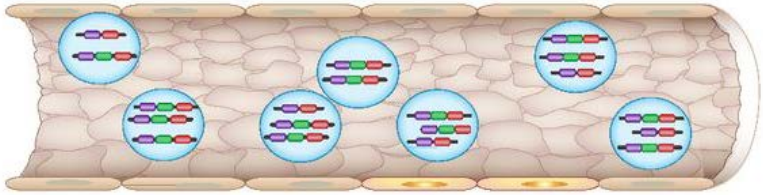
Genetic regulation of megakaryocytes and platelet

State of the art

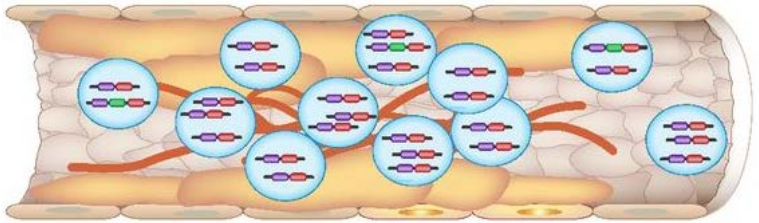
The genetic code of Megakaryocytes and Platelets Tells a Rich Story

Feature identified by RNA-seq	Molecular function
5′ cap and poly-A tail	Regulation of translation and RNA stability
Unspliced pre-mRNA and intron retention	Regulation of translation
Extended and alternate 5′ and 3′ UTR	Alternative regulatory motifs: uORFs, RNA binding proteins, and miRNA binding sites
Known and novel alternative splice variants	Regulation of translation, alternate functional protein isoforms, truncated proteins (i.e. secreted *vs.* membrane bound)
Non-coding RNA and antisense transcripts	Regulation of transcription and translation
Insertions, deletions, single nucleotide mutations	Altered functional proteins, altered protein expression

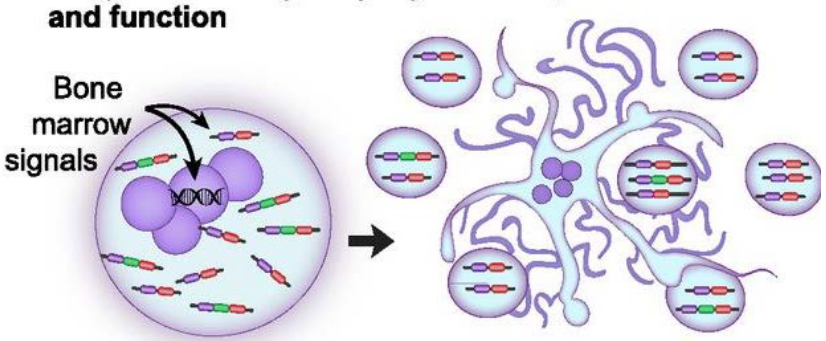
1. **Insights into platelet function and phenotype**



2. **Biomarkers and regulators of disease**



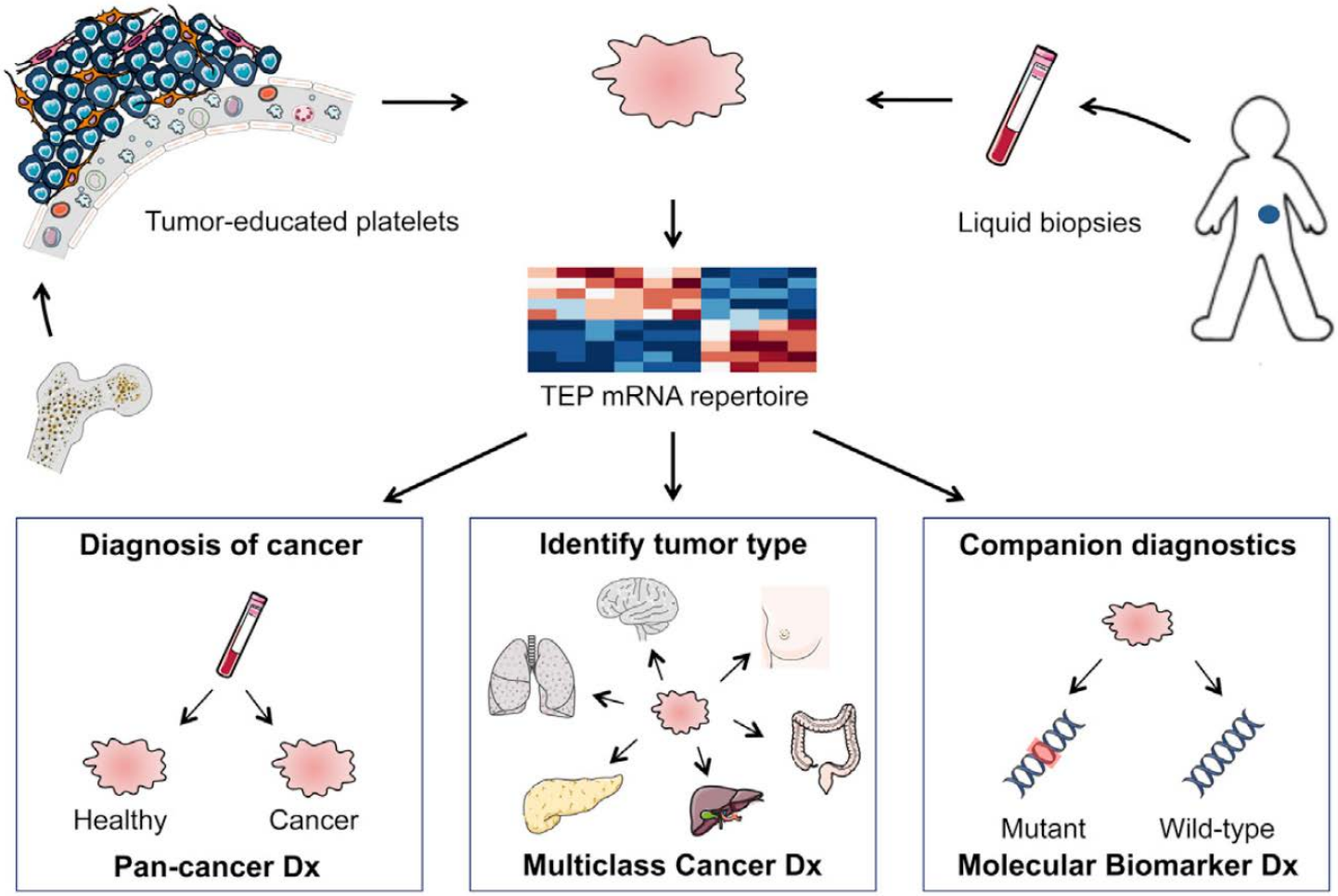
3. **Insights into megakaryocyte development and function**



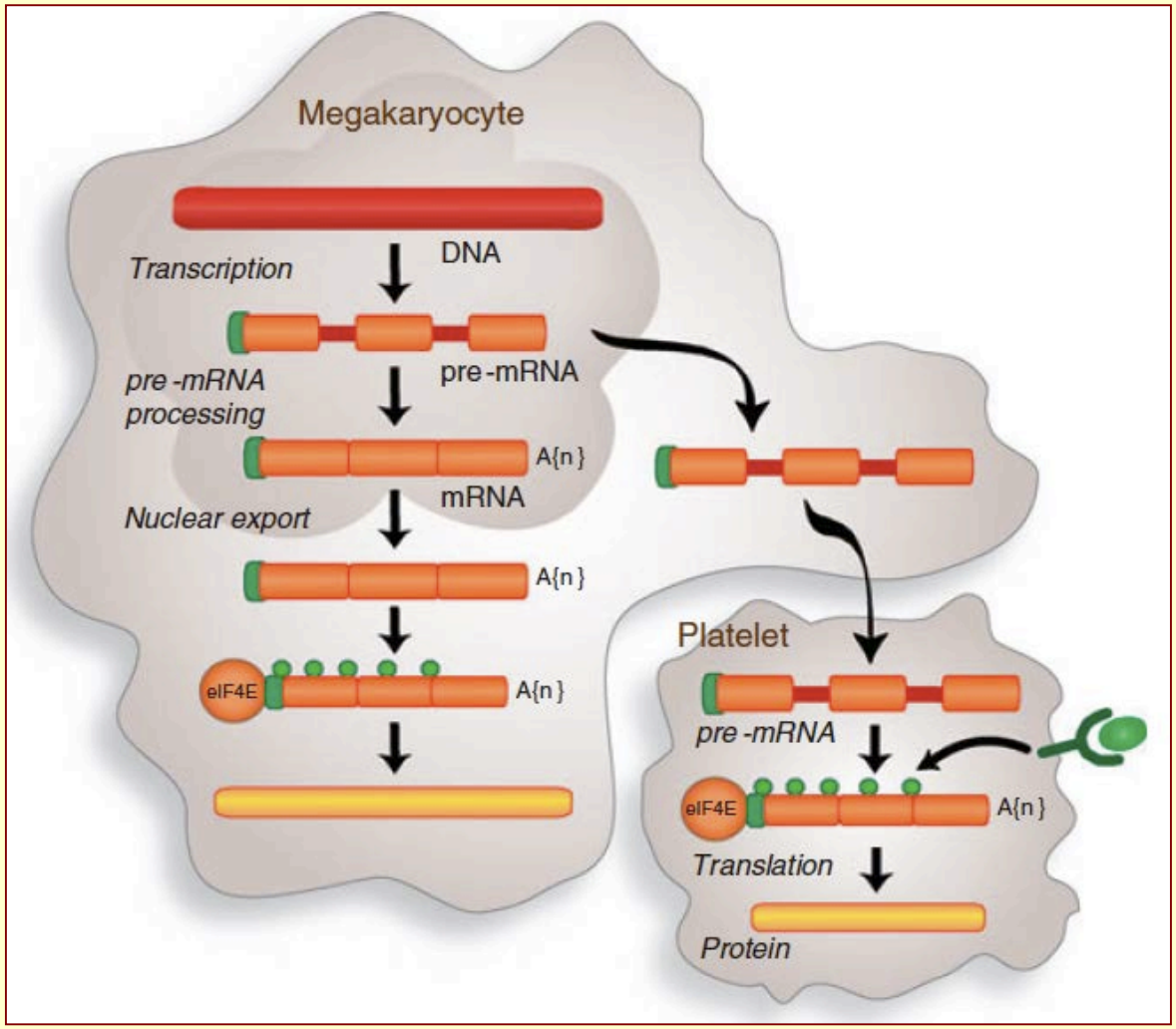
RNA signatures in platelets from cancer patients

Thomas Wurdinger
(SSC Platelet Physiology)

Tumors "educated" platelets (TEPs) by altering the platelet RNA profile



Protein synthesis by platelets



- Platelets do not contain DNA
- Platelets contain mRNA derived from megakaryocytes
- They are able of protein synthesis, constitutively or after stimuli

Weyrich AS et al. J Thromb Haemost 2009