MICROPARTICLES AND FIBRINOLYSIS
Micro-Particles and Fibrinolysis

- Are two major biological systems objectivating and regulating the state of body’s functions.

- Microparticles, consequence and cause of disease, contribute to its « evolution ».

- Fibrinolysis is a « multi-function » system, of difficult laboratory evaluation, involved in: brain/knowledge; fertility; malignancy; thrombosis/reperfusion.
Fibrinolysis Functions

- Neurology (brain)
- Malignancy (metastasis)
- Fertility
- Cell Remodelling
- Thrombosis
Fibrinolysis is a key system in life, probably still under evaluated.

Important (but occult?) function in regulating many biological functions.

Diagnostic and prognostic value for the major parameters (tPA, PAI-1, uPA, uPAR…).

Diagnostic potential of other factors (TAFI, PAI-2, MMPs, TIMPs,…).
Blood Clot: The Fibrinolysis Target
FIBRINOLYSIS IN BODY

- **Intra-vascular:** Mainly triggered by tPA

  - Plasmin $\Rightarrow$ Clot dissolution
  
  Body defence against thrombosis, Recanalisation, Thrombolytic therapy.

- **Extra-vascular:** Mainly triggered by uPA

  - Plasmin $\Rightarrow$ MMPs
  
  Matrix degradation and Tissue Remodelling or Neovascularisation (Cancer/Metastasis, Fertility, Cognitive functions of brain).
FIBRINOLYSIS REGULATION

Highly regulated biological system
- Early Progenitors release tPA
- Cells in later stages secrete uPA

Equilibrium between Activators and Inhibitors
- Intravascular: tPA-uPA/PAI-1, Plasminogen/α2AP/TAFI/HRGP/, MMP2-MMP-9/TIMP1-2
- Extravascular: uPA-uPAR/PAI-1, MMPs/TIMPs

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FIBRINOLYSIS ACTIONS

Extra-vascular

Intra-vascular

- tPA
- Pm
- Plg
- MMPs
- TIMPs
- PAI-1
- uPA
- uPA-R
- Clot
- TAFI
- α2AP/Plg
- HRGP
- Pm-α2AP
- PAI-1
- uPA
- PAI-1

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Schema of Fibrinolysis
tPA concentration in the micro-environment and in blood circulation

- tPA
- PAI-1
- α2AP
- α2M
- C1-INH

Clot

Trace Amounts
Free tPA

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PAI-1 in blood vessels

uPA → tPA → PAI-1

PAI-1 (VTN) (liver) → PLT

PAI-1 (IN) → tPA-PAI-1 → uPA-PAI-1 → Latent PAI-1

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MAJOR DIAGNOSTIC FIBRINOLYSIS ANALYTES

INTRAVASCULAR (PLASMA)
- tPA
- PAI-1
- uPA
- MMP-2
- MMP-9
- TIMP-1

EXTRAVASCULAR (Tissues)
- uPA
- uPA-R
- PAI-1
- MMPs/TIMPs
FIBRINOLYSIS IN BRAIN

- tPA involved in knowledge and protects from Alzheimer disease (tPA knock out mice model).
- When excessive in brain, can contribute to matrix degradation and aneurysm.
- Reactive fibrinolysis to cerebral thrombosis contributes to brain damage in stroke.
Yin and Yan effect of tPA in brain

Matrix degradation (negative)

Reperfusion (positive)

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Clinical applications of Fibrinolysis

- Metabolic Syndrome (X-Syndrome)
- Diabetes, Type II (not affected by Type I)
- Cardiovascular diseases (predictive value of tPA, PAI-1?, ...)
- Malignancy (Breast Cancer, ...), etc ...
ISSUES IN EVALUATING FIBRINOLYSIS

- It is a site targeted activity, promptly inhibited out of this location.
- Promoted and inhibited by locally secreted factors, present at high concentrations « only at these sites ».
- Very low residual active factors in blood circulation, and at low concentrations.
Microparticles as diagnostic markers

Endothelium

Platelets
R B C

W B C

Hypercoagulability
Inflammation
Infection
Malignancy

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Form AH100
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Microparticles

- Long shelf life ($\approx 6$ days)
- Bind to Annexin V
- Released from many blood cells
- Bear CDs, TF, TM, GP IÎ±-IIIa, ...
Cellular origin of microparticles

- **Platelets** (activation of coagulation)
- **Endothelial cells** (auto-immune diseases, TTP, activation of coagulation)
- **Monocytes** (inflammation, infection, …)
- **Leucocytes** (inflammation, …)
- **Lymphocytes** (diabetes mellitus, …)
- **Tumoral cells**
GENERATION OF MICROPARTICLES

Vessel

- WBC
- RBC
- Plt
- TRIGGER
- μp
- EC
- Plt

DISEASE

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Platelet activation

Resting

Activated
Platelet activation

Resting Platelet  Activation  Adhesion and spreading
Haemostasis and cell membrane remodeling

Microparticles = in vivo cell activation markers

Stimulus

Pro-inflammatory, Pro-apoptotic, Procoagulant...

Vesiculation

MP

Thrombin

[Ca^{2+}]_i

Ca^{2+}

Ca^{2+}

Cytoskeleton proteolysis

« flippase » activity

« floppase » activity

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Characteristics of MPs

- The general consensus is that MPs are small: 0.1 to 1µm.
- Microparticle membranes consist mainly of lipids and proteins.
- Expose the anionic phospholipids: PS.
- Express membrane antigens that reflect their cellular origin and the cellular processes triggering their formation.
PATHOLOGICAL MICROPARTICLES

- Myocardial infarction
- Diabetes
- Cancer
- Paroxystic Haemoglobinemia
- Hypertension
- Acute Coronary Syndrome
- type I Diabetes
- Lupus Anticoagulant
- HIV
- Sepsis
- Preeclampsia
- type II Diabetes

Martinez et al., Am. J. Physiol (2005)
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Microparticles

Cause and consequence of disease states

DISEASE

Procoagulant
Pro-inflammatory
Clinical usefulness of MPs study

- Modulate the Hemostatic balance and can cause its disruption.
- Procoagulant MPs in Immune-mediated Thrombosis.
- Procoagulant MPs in Atherothrombosis.
- Angiogenesis and MPs.
- Circulating MPs: Effectors in the Tuning of Thrombotic Propensity Associated with Cardiovascular Risk.
- Pharmacological Modulation of Circulating MPs.
Clinical applications of MPs

- Prognosis of myocardial infarction.
- Follow-up and therapy monitoring of patients with myocardial infarction.
- Prognosis of recurrence risk.
- Diabetes, Malignancy, Pregnancy.
Pathological variations of microparticles

- Elevated in M.I. (x2 to x10)
- Elevated in cancer (predictor of metastasis?)
- When Elevated, can predict vascular complications in diabetes
- Elevated in haemophilia (x10)
- During Novoseven (VIIa) therapy
- Correlates with severity of hypertension

Note: responds to therapy efficacy
MPs can be measured in atherosclerotic plaques...

... and in Blood!
Evaluation of Microparticles

**Flow Cytometry:**
- Only « large microparticles (> 0.4 µ) are measured (size, content).
- Characterised by antibody/label used.

**Activity/Immuno-Assay:**
- All MPs are measured (including < 0.4 or 0.1 µ)
- Measurement of associated procoagulant activity (PS equivalent).
- Identification of cell origin with MoAbs.
Microparticle measurement

Different methodologies are available for MPs determination:

- Flow cytometry relies on the antigenic composition of MPs and allows them to be enumerated according to their cellular origin.

- ELISA capture with Annexin V or antibody and determination of procoagulant activity of MPs.
Microparticles in Fibrinolysis

- Elevated PAI-1 induces an important release of endothelial MPs with procoagulant activity.
- Cancer cells release microparticles exposing TF, or uPAR-uPA.
- Chemotherapy generates microparticles from tumoral cells, possibly inducing fibrinolysis (ovarian, prostatic malignancies, acute promyelocytic leukemia,...) and/or thrombosis.
Endothelial MPs
Fibrinolytic Markers and Microparticles in Cancer

- Cancer cells promote fibrinolysis for migrating, producing metastasis (uPAR-uPA and MMPs mediated).
- Fibrin protects from tumor growth, but also protects malignant cells from host defence.
- Fibrin attracts EC and favors angiogenesis.
- Cancer cells generate TF and MPs exposing TF, inducing « hypercoagulability ».
Emerging markers of tumor invasion

They bring complementary information on disease activity to usual cancer markers:

- **Measured in tissue extracts:**
  - uPA
  - PAI-1
  - uPA-PAI-1 complexes
  - TF
  - Breast Cancer, many tumors
  - Many Tumors (lung, panceatic, gastric,....)

- **Measured in plasma:**
  - MMP-2
  - MMP-9
  - TIMP-1
  - MMP-9-TIMP complexes
  - Tumors invasiveness

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Conclusions

- Fibrinolysis and Micro-Particles are emerging or «rediscovered» body’s functions with multiple impacts and implications in diseases.
- Their laboratory exploration can contribute to management of pathology and therapy monitoring.
- Understanding their mechanisms of action is useful for new drug developments.
- High potential, in clinical practice, provided that pre-analytical variables are well controlled.