PHOSPHOINOSITIDES, CYTOSKELETON AND PLATELET ACTIVATION

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CONTEXT

I] Role of PI3K p110β and its products in platelet activation: Our previous results suggested that the class IA phosphoinositide 3-kinase (PI3K) p110β play a major role in platelet activation. To evaluate the potential interest of this isoform of PI3K as a new target for antithrombotic therapy, we generated a mouse model with a PI3K p110β inactive catalytic subunit specifically in megakaryocytes and platelets (*PF4*-Cre/p110β$^{lox/lox}$).

II] Role of SHIP1 in cytoskeleton organization:
Phosphoinositide phosphatases involved in the hydrolysis of PtdIns(3,4,5)P_3 (PIP3) and PI(3,4)P_2 are essential for the negative regulation of the 3-phosphoinositides (3-PIs) signaling pathway. In platelet, the termination of the PI3K signaling by degradation of PIP3 is mediated by the Src-homology 2 (SH2) domain-containing inositol 5-phosphatases (SHIP1).
We have previously shown that SHIP1-deficient platelets have large membrane extensions, abnormalities in the open canalicular system and a dramatic decrease in close cell-cell contacts. Interestingly, SHIP1 appeared to be required for platelet contractility and subsequent thrombus organization and fibrin clot retraction.

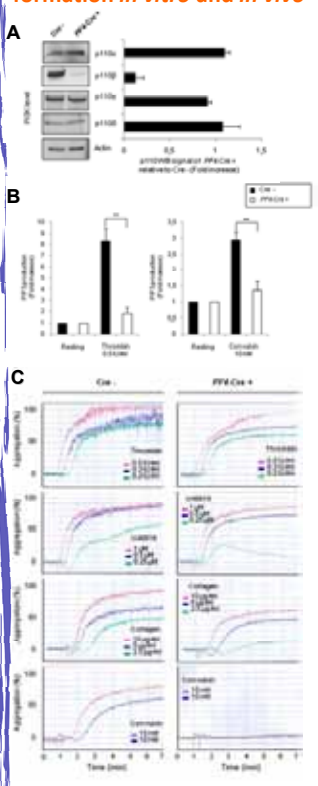
OBJECTIVES

I] Role of PI3K p110β and its products in platelet activation
1- Generation of the mouse model with an inactive PI3Kβ in megakaryocyte lineage to prove the importance of PI3Kβ in platelet activation and as potential target for anti-thrombotic drugs.
2- Role of PI3K p110β and its products in platelet activation
a) Signaling mechanism affected by PI3Kβ defect: Our first aim has been to evaluate the participation of PI3Kβ in the 3-PI metabolism.
b) Functional implication of PI3Kβ on platelet activation: The consequence of the inactivation of PI3Kβ in platelet responses and thrombus growth has been evaluated *in vitro* and *in vivo* in physiopathological schemes.

II] Role of SHIP1 cytoskeleton organization: We investigated in detail SHIP1-deficient platelet cytoskeleton organization and acto-myosin association using immunostaining approach of cytoskeleton components of platelets in suspension and classical biochemistry approaches.

RESULTATS

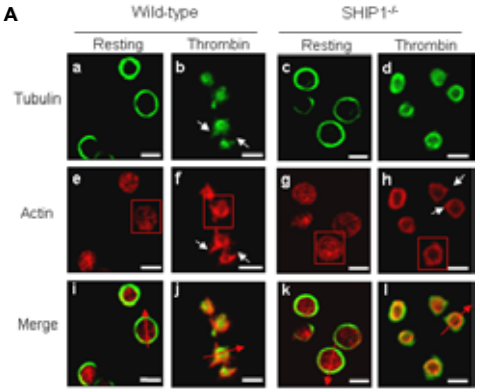
Deletion of the p110β isoform of PI3K in platelets reveals its central role in PIP3 production and thrombus formation *in vitro* and *in vivo*



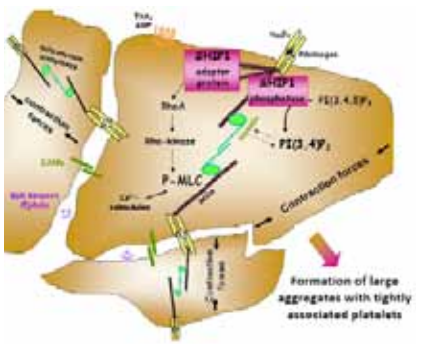
Martin V, Guillermet-Guibert J, Chicanne G, Jandrot-Perrus M, Vanhaesebroeck B , Plantavid M, Payrastre B, Gratacap M-P. *Deletion of p110 beta isoform of phosphoinositide 3-kinase in platelets reveals its central role in heterotrimeric G protein and ITAM-signaling*. **Blood,** 2010

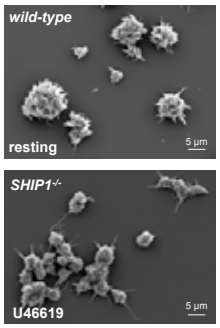
A) Conditional genetic inactivation of p110β in the megakaryocytic lineage has no impact on the expression of other class I PI3K catalytic subunits (p110α, p110γ and p110δ) ***B)*** PI3Kβ is critical for PIP3 production downstream of both GPCR and GPVI/Tyrosine kinases signalling. Deletion of p110β affects functional platelet responses *in vitro* ***(C)*** and *in vivo* ***(D)*** after $FeCl_3$ induced carotid artery injury.

SHIP1 : role in platelet activation and thrombus growth *via* the control of cytoskeleton dynamics



S. Séverin, G. Chicanne, V. Martin, S. Allart, A. Eckly, M. Plantavid, B. Payrastre, M-P. Gratacap. *A role for SHIP1 in platelet internal contraction via the control of actomyosin and microtubule dynamics.* 2011, submitted.





A) Cytoskeletal reorganization abnormalities in SHIP1-deficient platelets. Wild-type or SHIP1 deficient platelets were stimulated with thrombin (0.5IU/ml, 2min) in suspension under non-aggregating conditions and fixed with paraformaldehyde. Representative confocal immunofluorescence images of platelet microtubules (green) and F-actin cytoskeleton (red) are shown. In merged images, yellow coloration indicates colocalisation of α-tubulin with F-actin. Data shown are representative of four independent experiments (scale bar = 2 µm) ***B)*** SHIP1 acts as a phosphatase and adapter protein: SHIP1 deficiency affects the thrombin-dependent membrane / cytoskeleton recruitment of myosin IIA *via* its phosphatase activity and RhoA activation *via* its adapter protein property ***C)*** SHIP1-deficient platelets form small aggregates (TxA2 analogue U46619 (0.5 µM), 3 min, scanning electron microscopy (bars 5 µm)).

CONCLUSION - PERSPECTIVES

Deletion of the p110β isoform of PI3K in platelets reveals its central role in Akt activation and its contribution to platelet responses

- PI3Kβ is critical for PIP_3 production and PKB/Akt activation downstream of both GPCR and GPVI/tyrosine kinases signalling.
- Functional studies showed an almost complete inability of these platelets to adhere onto fibrinogen under flow condition suggesting that PI3Kβ is also acting downstream of $\alpha_{IIb}\beta_3$ (not shown).
- *In vivo* studies showed that these mice have a normal bleeding time but are resistant to thrombosis after $FeCl_3$ injury of the carotid. However we noticed a tendency to generate platelet emboli, a potential deleterious phenomenon. Our current research is focus in a better characterization of this phenomenon (Martin et *al.*, in preparation).

Role of SHIP1 cytoskeleton organization

- SHIP1 plays an important role in the control of internal platelet contraction by regulating platelet cytoskeleton reorganization during stimulation in suspension, in the absence of integrin engagement but is not directly involved in actin polymerization. Interestingly, we found that SHIP1 is required for full activation of RhoA, MLC phosphorylation and stable association between myosin IIA and the platelet cytoskeleton. These effects may be due to the docking protein properties of SHIP1 since RhoA activation is independent of 3-PIs formation.
- Together, these results reveal new functions for SHIP1 as a key regulator of cytoskeletal reorganisation required for internal contractile activity, an important feature of platelet activation and thrombus growth (Severin et *al.*, submitted). Our current research is focus in a better characterization of SHIP1 partners.

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Université Paul Sabatier Toulouse III

PUBLICATIONS - VALORISATION

Articles
- V. Martin, B. Hechler, G. Chicanne, J. Guillermet-Guibert, B. Vanhaesebroeck, B. Payrastre, M-P. Gratacap. *PI3Kb is essential for platelet thrombus stabilization ex-vivo and in vivo.* In preparation.
- S. Séverin, G. Chicanne, V. Martin, S. Allart, A. Eckly, M. Plantavid, B. Payrastre, M-P. Gratacap. *A role for SHIP1 in platelet internal contraction via the control of actomyosin and microtubule dynamics.* 2011, submitted.
- Martin V, Guillermet-Guibert J, Chicanne G, Jandrot-Perrus M, Vanhaesebroeck B , Plantavid M, Payrastre B, Gratacap M-P. *Deletion of p110 beta isoform of phosphoinositide 3-kinase in platelets reveals its central role in heterotrimeric G protein and ITAM-signaling.* **Blood,** 2010 Mar 11;115(10):2008-13.
- Gratacap M-P, Martin V, Valéra M-C, Allart S, Garcia C, Sié P, Recher C, Payrastre B. *The new tyrosine-kinase inhibitor and anti-cancer drug dasatinib reversibly affects platelet activation in vitro and in vivo.* **Blood.** 2009; 27;114(9):1884-92.
- Carrié D, Garcia C, Gratacap M-P, Voisin V, Payrastre B, Sié P. *Assessment of platelet response to clopidogrel through measurement of ADP-induced Akt phosphorylation.* **J. Thromb. Haemost**. 2009, 7(8):1411-3.

Revues
- Gratacap MP, Guillermet-Guibert J, Martin V, Chicanne G, Tronchère H, Gaits-Iacovoni F, Payrastre B. *Regulation and roles of PI3Kb, a major actor in platelet signaling and functions.* **Adv Enzyme Regul**. 2010 Oct 28
- Gratacap M-P, Séverin S, Chicanne G, Plantavid M, Payrastre B. *Different roles of SHIP1 according to the cell context: the example of blood platelets*. **Adv Enzyme Regul.** 2008;48:240-52.

Communications
- Valérie Martin, Marie-Pierre Gratacap, Christian Recher, P. Sié, Gaëtan Chicanne, Marie-Cécile Valéra, Julie Guillermet-Guibert, Bart Vanhaesebroeck & Bernard Payrastre: Platelet signaling and functions in the absence of src-kinases or PI 3-kinase β activities. **Nottingham Platelet Conference**, July 2010, Nottingham, UK
- S. Séverin, G. Chicanne, V. Martin, S. Allart, A. Eckly, J. M. Penninger, M. Plantavid, B. Payrastre, M-P. Gratacap: *The SH2 domain containing inositol polyphosphate 5-phosphatase SHIP1 : role in platelet activation and thrombus growth.* **Enzyme and cell regulation,** 2010, Bologne, Italy
- S. Séverin, G. Chicanne, V. Martin, S. Allart, A. Eckly, J. M. Penninger, M. Plantavid, B. Payrastre, M-P. Gratacap : *A role for SHIP1 in platelet internal contraction via the control of actomyosin and microtubule dynamics.* **International Society on Thrombosis and Haemostasis (ISTH),** XXII Congress, July 11-16 2009, Boston, USA.
- V. Martin, J. Guillermet-Guibert, G. Chicanne, M. Jandrot-Perrus, M. Plantavid, B. Vanhaesbroeck, B. Payrastre, M-P. Gratacap: *Deletion of the p110β isoform of phosphoinositide 3-kinase in platelets reveals its central role in Akt activation and its contribution to platelet response :* **French-UK platelets meeting**, octobre 2009, Toulouse, France