LANDSCAPE

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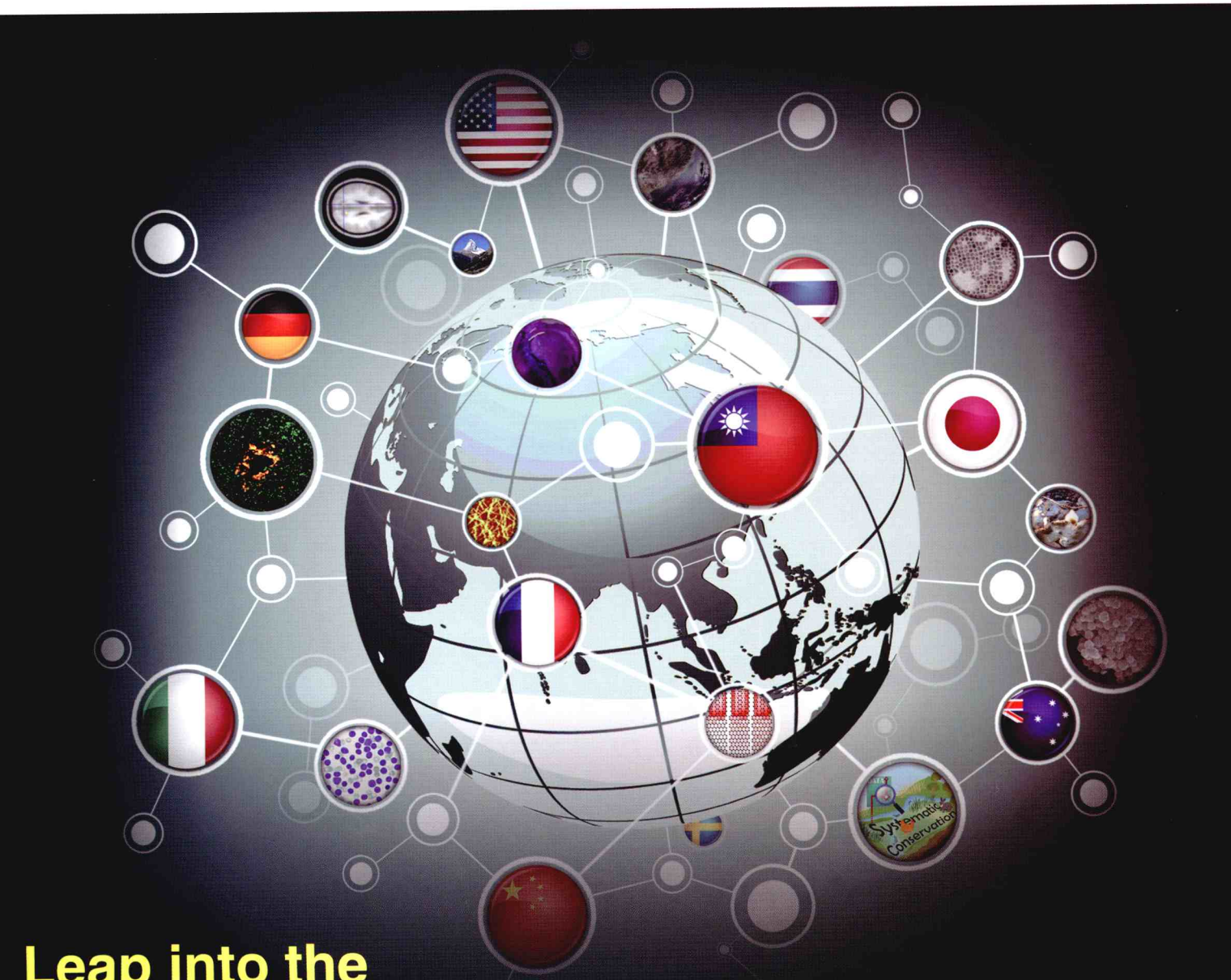

Leap into the Cutting-edge Terrain of Internationalization

After Adoption: Bridging a Way to Their Origins

DynaPho: A Phospho-Tool in Systems Biology

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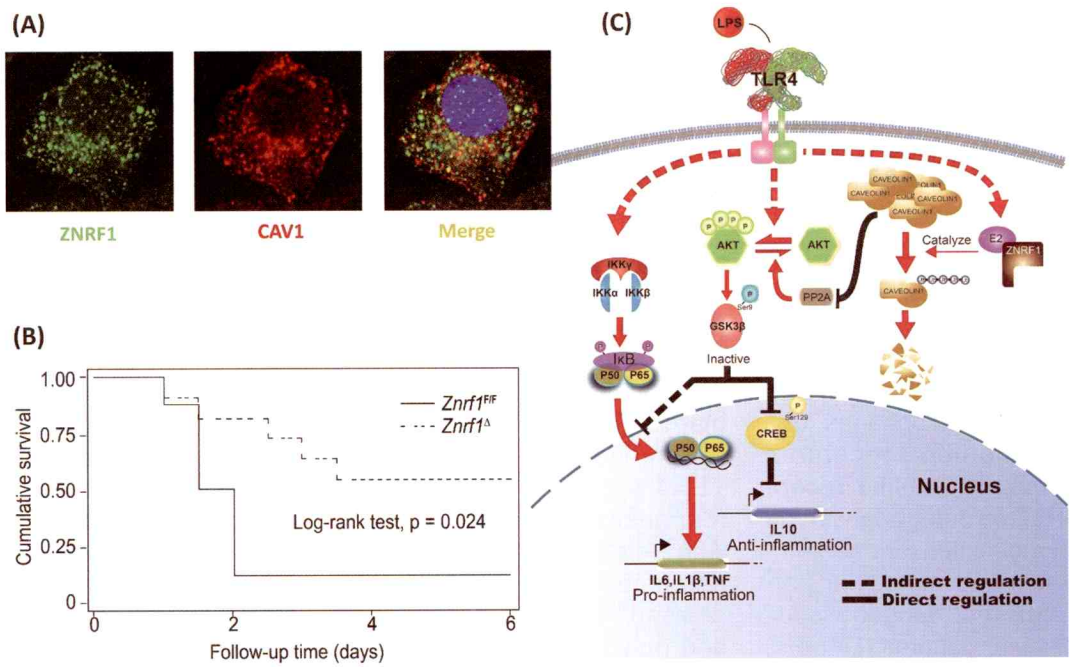

Figure 1. (A) Immunofluorescence staining showing that ZNRF1 partially colocalizes with Caveolin-1. (B) The survival of Znrf1$^{\Delta}$ mice (dotted line, n=8) was longer than that of Znrf1$^{F/F}$ mice (solid line, n=8) after cecal ligation and puncture. (C) The proposed model summarizing the control of Caveolin-1 protein levels and TLR-triggered immune responses by ZNRF1.

Snake venom is a key ingredient for developing anti-thrombotic agents

Upon vessel wall injury, the integrity of the endothelial cell layer is disrupted, and platelets interact with exposed extracellular matrix (ECM) molecules. The initial interaction between platelets and subendothelial collagens under high-shear conditions is indirectly mediated by a plasma protein, von Willebrand factor (vWF), which binds collagen and the platelet glycoprotein (GP) Ib/V/IX complex. This unstable interaction facilitates transient platelet tethering and rolling on the injured endothelial cells. Subsequent firm adhesion is mediated by collagen binding to its platelet receptors, principally integrin α2β1 and GPVI. Recently, elevated platelet GPVI expression was found in acute coronary syndromes, transient ischemic attacks and strokes, indicating that GPVI may serve as a biomarker for acute atherothrombotic events. Using Fab fragments from monoclonal antibodies effectively inhibits collagen-induced platelet aggregation in vitro and ex vivo and during in vivo thrombosis in rats without prolonging the bleeding time. An increasing number of studies imply that blocking collagen from binding to GPVI prevents initial adhesion and further activation of the platelets and has an enormous impact on antithrombotic therapy.

GPVI is an important collagen receptor and an ideal target for snake venom proteins. When GPVI is clustered by ligands, it induces massive platelet activation. Several snake venoms that activate platelets via GPVI have been identified, including trowaglerix from *Tropidolaemus wagleri*. The partial amino acid sequences of trowaglerix were used to demonstrate that the hexa-/decapeptides of its α subunit specifically exhibited marked inhibitory activity against collagen-induced platelet aggregation by interacting with the GPVI receptor in vitro and effective anti-thrombotic activity in mouse models without causing bleeding. Through computational peptide design and molecular

dynamics simulations of the trowaglerix-based decapeptides and GPVI, a possible binding site was identified near the D1/D2 domain surface, which differs from well-known collagen-binding sites.

To the best of our knowledge, this is the first study to design small-mass peptides derived from snaclecs with anti-thrombotic activity that target the platelet GPVI receptor with an atypical binding epitope. We also demonstrated the in vivo antithrombotic effect of trowaglerix-based decapeptides. Intravenous administration of trowaglerix-based decapeptides delayed thrombus formation and significantly prolonged time-to-occlusion (TTO) in irradiated mesenteric venules of mice pretreated with fluorescein sodium. Prolonged bleeding is a common undesirable side effect of antithrombotic therapy. Trowaglerix-based decapeptides did not noticeably affect the bleeding time in mouse tail transection models. These results provide a promising novel molecular skeleton for designing a new class of anti-thrombotic agents targeting platelet GPVI with limited bleeding side effects.

Since all of the natural ligands of GPVI are proteins, new agents should be developed as peptide modulators that can regulate protein-protein interaction. In addition, peptide drugs possess many advantages, such as high potency and specificity with few toxicological problems. To find better decamer peptides that inhibit GPVI on the collagen-binding site, we developed a greedy algorithm-based peptide-design method (Figure 1). The analysis of the structure-activity relationship will provide valuable information for exploring many facets of platelet function and hemostasis. We will further study the linear peptide, cyclic peptide and small molecule compound to provide new options to further treat arterial thrombogenic diseases.

(A)

Sequence	Inhibition at 100μg/ml	Docking Score (Kcal/mol)
GFCKWMNVAC	93.75±6.25	-6.1
LFHVWPYWWW	34.75±13.51	-7.7
LFHLWPYWWW	21.47±15.90	-6.7
LFHVWDYYDR	60.52±22.62	-6.7
LFHVWDYTDR	95.18±4.83	-6.5

(B)

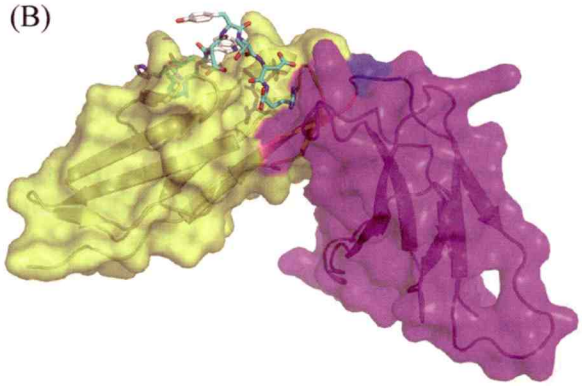

Figure 1. The docking scores on the collagen-binding site of GPVI, platelet aggregation inhibition activity of selected decamer peptides from trowaglerix α (A) and the docked pose of peptide LFHVWDYTDR (cyan) on the collagen-binding site of GPVI (B).

Reference

Chien-Hsin Chang, Ching-Hu Chung, Yi-Shu Tu, Cheng-Chieh Tsai, Chun-Chieh Hsu, Hui-Chin Peng, Yufeng J. Tseng, and Tur-Fu Huang (2017). Trowaglerix venom polypeptides as a novel antithrombotic agent by targeting immunoglobulin-like domains of glycoprotein VI in platelet. *Arteriosclerosis, Thrombosis, and Vascular Biology, 37*(7), 1307-1314. DOI:10.1161/ATVBAHA.116.308604

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