XL JCET LECTURE, 11.07.2014, 9.00

Regulatory mechanism of fibrinolytic activity in plasma and on vascular endothelial cells (VECs): Unique secretory dynamics of tissue plasminogen activator and the expression of its activity on VECs

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Vascular endothelial cells (VECs) contribute to keep the patency of vasculature by expressing anti-coagulatory and pro-fibrinolytic activities. Tissue-type plasminogen activator (tPA), an enzyme which catalyzes the initial step of fibrinolysis, is secreted from VECs as an active form and directly enhances fibrinolytic potential. Recently, we succeeded to visualize its secretory dynamics in GFP-tagged tPA (tPA-GFP) expressing VECs using total internal reflection fluorescence microscopy. tPA-GFP appeared to have a unique secretory dynamics and to remain on the cell surface after exocytosis from its secretory granules. Studies using mutants of tPA-GFP suggested that the binding to the cell surface heavy-chain- as well as catalytic activity-dependent. The retained active tPA-GFP on cell-surface effectively activated plasminogen, which accelerated further accumulation of plasminogen on cell surface as well as intercellular/matrix adhesive area, and effectively lysed fibrin network formed on VECs. PA inhibitor-1 (PAI-1) facilitated dissociation of cell surface-retained tPA-GFP by forming a high molecular weight complex, and suppressed the expression of fibrinolytic activity. Thus, PAI-1 appeared to control fibrinolytic activity not only in plasma but also on the surface of VECs. We also analyzed micro-thrombus formation and its lysis on laser-induced injury site of VECs using intra-vital confocal microscopy. These processes appeared to be well controlled by spatiotemporal regulatory system of platelets activation as well as the activation of coagulation cascade. The involvement of fibrinolytic system from the very early phase of thrombus formation was also proved. In addition to these our experimental data, I also demonstrate two distinct cases of genetically characterized PAI-1-deficient Japanese subject, and discuss their molecular bases as well as unique phenotypes which are largely different from those of PAI-1 deficient mice.

CURRICULUM VITAE

Tetsumei Urano, MD, PhD
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Born: February 02, 1956

Place of Birth: Mie, Japan

Nationality: Japanese

Sex: Male

Marriage Status: Married

Education & Occupation:

Finished Ise High School, March, 1974.
Admitted to Hamamatsu University School of Medicine, April, 1975.
Finished same March, 1981.
Admitted to Graduate School, Hamamatsu University School of Medicine (Majored in Surgery and Physiology), April, 1981.
Passed 71st National Examination for Medical Practitioners and received license for medical practice (#257349), May 1981.
Graduated from Graduate School of Hamamatsu University School of Medicine, and received Doctor of Medical Science, March, 1985.
Belonged to the Second Department of Surgery (Prof. S. Sakaguchi) of Hamamatsu University School of Medicine, April, 1985.
Joined Dr. Castellino's Laboratory in University of Notre Dame in USA as a post doctoral fellow, July, 1986.
Finished post doctoral course in the same laboratory, August, 1988.
Belonged to the Second Department of Physiology (Prof. A. Takada) of Hamamatsu University School of Medicine as an assistant, August, 1988.
Joined Dr. Tor Ny's Laboratory as a visiting scientist sponsored by Japanese Government, June, 1990.
Joined Dr. Sixtus Thorsen's Laboratory as a visiting scientist sponsored by Japanese Government, March, 1991.
Associate Professor in Department of Physiology, Hamamatsu University School of Medicine, October, 1991.
Professor in Department of Physiology, Hamamatsu University School of Medicine, April, 2001.

Selected publications:

1. Urano T, Castellino FJ, Ihara H, Suzuki Y, Ohta M, Suzuki K, Mogami H. Activated protein C attenuates coagulation-associated over expression of fibrinolytic activity by suppressing the thrombin-dependent inactivation of PAI-1. Journal of Thrombosis and Hemostasis 1, 2615-2620, 2003

2. Zhao BQ, Ikeda Y, Ihara H, Urano T, Fan WY, Mikawa S, Suzuki Y, Kondo K, Sato K, Nagai N and Umemura K. Essential role of endogenous tissue plasminogen activator through matrix metalloproteinase 9 induction and expression on heparin-produced cerebral hemorrhage after cerebral ischemia in mice. Blood 103(7), 2610-2616, 2004
3. Nakamura R, Umemura K, Hashimoto H, Urano T. Less pronounced enhancement of

thrombin-dependent inactivation of plasminogen activator inhibitor type 1 by low molecular weight heparin compared with unfractionated heparin. Thromb Haemost 95, 637-42, 2006

4. Hayashi T, Mogami H, Murakami Y, Nakamura T, Kanayama N, Konno K, Urano T. Real-time analysis of platelet aggregation and procoagulant activity during thrombus formation in vivo. Pflugers Archiv-European Journal of Physiology 456(6), 1239-51, 2008

5. Suzuki Y, Mogami H, Ihara Y, Urano T. Unique secretory dynamics of tissue plasminogen activator and its modulation by plasminogen activator inhibitor-1 in vascular endothelial cells. Blood 113, 470-478, 2009

6. Suzuki, Y and Urano T. Novel mechanism of the expression and amplification of cell surface-associated fibrinolytic activity demonstrated by real-time imaging analysis. Journal Pharmacological Sciences 116(1), 19-24, 2011

7. Rybaltowski M, Suzuki Y, Mogami H, Chlebinska I, Brzoska T, Tanaka A, Banno F, Miyata T, Urano T. In vivo imaging analysis of the interaction between unusually large von-Willebrand factor multimers and platelets on the surface of vascular wall. Pflugers Arch - Eur J Physiol 461(6), 623-633, 2011

8. Iwaki T, Tanaka A, Miyawaki Y, Suzuki A, Kobayashi T, Takamatsu J, Matsushita T, Umemura K, Urano T, Kojima T, Terao T, Kanayama N. Life-threatening haemorrhage and prolonged wound healing are remarkable phenotypes manifested by complete PAI-1 deficiency in humans. Journal of Thrombosis and Haemostasis 9(6), 1200-1206, 2011

9. Suzuki Y, Yasui Y, Brzoska T, Mogami H, Urano T. Surface-retained tPA is essential for effective fibrinolysis on vascular endothelial cells. Blood 118(11), 3182-3185, 2011

10. Nishimura S, Manabe I, Nagasaki M, Kakuta S, Iwakura Y, Takayama N, Ooehara J, Otsu M, Kamiya A, Petrich B, Urano T, Kadono T, Sato S, Aiba A, Yamashita H, Sugiura S, Kadowaki T, Nakauchi H, Eto K, and Nagai R. In vivo imaging visualizes discoid platelet aggregations without endothelium disruption and implicates contribution of inflammatory cytokine and integrin signaling. Blood **119**(8): p. e45-56, 2012

11. Kramkowski K, Leszczynska A, Mogielnicki A, Chlopicki S, Fedorowicz A, Grochal E, Mann B, Brzoska T, Urano T, Motterlini R, Buczko W. Anti-thrombotic properties of water-soluble carbon monoxide-releasing molecules (CO-RMS). Arterioscler Thromb Vasc Biol **32**, 2149-2157, 2012

12. Brzoska T, Suzuki Y, Mogami H, Sano H, Urano T. Binding of thrombin-activated platelets to a fibrin scaffold through $\alpha_{IIb}\beta_3$ evokes phosphatidylserine exposure on their cell surface. Plos One, 8: E55466, 2013