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Regulatory proteins and processes in blood: Kinases, vitronectin, PAI-1 and PEDF

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Our research is aimed recently at the elucidation of regulatory proteins and processes involved in the concerted biological functions in blood. In the last two years we focused on protein kinases and their role in the modulation of cell adhesion via vitronectin (Vn), on plasminogen-activator inhibitor-1 (PAI-1) and on the pigment epithelium derived factor (PEDF). Since de-regulation of such processes causes many of the major human diseases (heart failure, stroke, retinopathies cancer, etc.), such studies will hopefully improve diagnosis and the design new drugs.

The PKA Phosphorylation of Vitronectin Affects its Function by Modulating its Conformation (1)

We have previously reported that Vn is specifically phosphorylated by PKA (at Ser378), a kinase we have previously shown to be released from platelets upon their physiological activation. We have recently elucidated some of the molecular consequences of this phosphorylation and showed (by circular dichroism, by phosphorylation with casein kinase II, and by the use of Vn mutants) that the PKA phosphorylation of Vn affects its function by altering its conformation. The evidence compiled suggests that this phosphorylation of Vn can modulate plasminogen activation and consequently control fibrinolysis.

Localization of Protein Kinase A and Vitronectin in Resting Platelets and their Translocation Onto Fibrin Fibers During Clot Formation (2)

In the last two years we showed that (i) intact platelets possess on their surface an ecto-PKA which can preferentially phosphorylate Vn; (ii) in the resting platelet, both the catalytic and the regulatory subunits of PKA are present on the platelet surface, in the surface connected canalicular system, and within the α-granules of the platelets; (iii) the process initiated upon platelet activation, which leads to the formation of fibrin fibers and consequently forms the fibrin net, is accompanied by a translocation of PKA, of Vn, and of PAI-1 onto the fibrin fibers (Fig. 1). We propose a mechanism through which plasminogen activators may trigger by means of the PAI-1-Vn system the formation of active plasmin to initiate fibrinolysis.

Vitronectin Binds to Fibrin and can thus Anchor to Blood Clots (paper submitted).

Vn constitutes together with fibrin and fibronectin the provisional matrix. Work done this year shows that Vn specifically interacts with fibrin, and that this interaction involves the amino-terminal edge (Asp1-Thr44) of this adhesion protein as well as its carboxy terminal domain (Lys348-Arg379). The binding of Vn to fibrin enhances the adhesion of endothelial cells and of platelets onto the fibrin net, but prevents the Vn phosphorylation by PKA. Our results suggest that Vn may interact with fibrin at wound healing areas, and that this interaction may enhance cell adhesion, an essential step in blood vessel repair.

Phosphorylation of Vitronectin by Casein Kinase II and the Promotion of Cell Adhesion via the $\alpha v\beta 3$ - PI3K pathway (3,4)

Phosphorylation of Vitronectin by Casein Kinase II was previously shown to occur at Thr50 and Thr57 and to augment a major physiological function of Vn: cell adhesion and spreading. We recently showed that this phosphorylation increases cell adhesion via the $\alpha v\beta 3$ but not via the $\alpha v\beta 5$ integrin, suggesting that $\alpha v\beta 3$ differs from $\alpha v\beta 5$ in its biorecognition profile. Whereas both the phospho and non-phospho analogs of Vn (mutants Vn(T50,57E), and Vn(T50,57A) respectively), trigger both the $\alpha v\beta 3$ and the $\alpha v\beta 5$ integrins and equally activate the MAPK pathway, these two forms are distinctly different in their activation of the PI3K - AKT/PKB pathway. The latter is shown to involve $\alpha v\beta 3$ but not $\alpha v\beta 5$, and to bring about an enhanced cell adhesion on Vn(T50,57E), representing the CKII phosphorylated Vn. The occurrence of a cell surface receptor that specifically distinguishes between a phosphorylated and a non phosphorylated Vn, together with the fact that it preferentially activates a distinct intra-cellular signaling pathway, suggest that extra-cellular CKII phosphorylation may well play an important role in the regulation of cellular processes such as adhesion.

Evidence showing that the two-chain form of vitronectin is produced in the liver by a selective furin cleavage (5)

Vitronectin (75 kDa) occurs in human blood fluid as a single chain (Vn75) or a two-chain form ($Vn65^{+}10$), and is produced by a specific cleavage, (at Arg379-Ala380) by a proteinase not identified hitherto. These two forms were shown to be

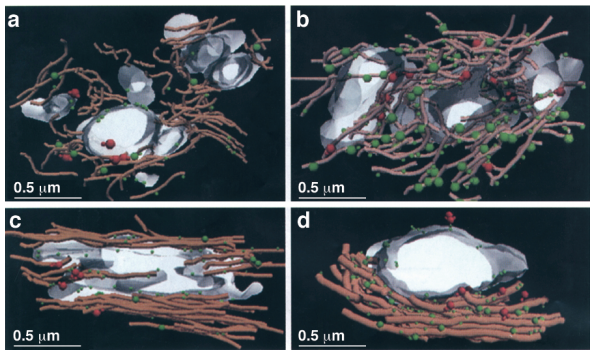

Fig. 1 *3-D presentation of the PKA and Vn colocalization in platelet clots. The larger spheres represent Vn labels, and the smaller spheres represent PKA. The red spheres stand for colocalized PKA and Vn (distance <10 nm), while the green spheres stand for non-colocalized PKA and Vn. Panels (a) and (b) represent a clot, 5 minutes after platelet activation. Panels (c) and (d) represent a clot 30 minutes after platelet activation.*

functionally different and therefore, this cleavage may have a regulatory significance *in vivo*. We recently used a tailored one-chain recombinant Vn, a specific PKA phosphorylation at Ser378, and sequence analysis to show (i) that none of the proteinases originating from blood, previously thought to be the endogenous proteinase (plasmin, thrombin, tPA, and uPA) is indeed the *in vivo* convertase; (ii) that furin, a serine endoproteinase residing in the secretory pathway of hepatocytes, where Vn is synthesized, specifically cleaves Vn at the endogenous cleavage site. Consequently, we propose that the Vn75 to Vn65^{+}10 conversion takes place in the liver (not in blood) and is carried out by furin.

The C-Terminal Tail of KSMP/Meprin β is Involved in the Intracellular Trafficking of this Membranal Proteinase (6 and paper submitted)

The Kinase Splitting Membranal Proteinase (KSMP) discovered and characterized in our laboratory, is identical to the β subunit of meprin - a metalloendoproteinase located in brush border membranes. Using truncated mutants of rat meprin β expressed in Cos-7 and HEK 293 cells, we showed here that the cytoplasmic tail of KSMP/meprin β is indispensable for its exit from the ER. A meprin β mutant lacking the last 25 amino acids is shown to be transport-incompetent, although it does not contain any of the known ER-retention signals. Systematic analysis of the rate of the ER-to-Golgi transport using a series of mutants with Ala or Pro substitutions in the tail, suggests that while no specific amino acid residue by itself is imperative for normal intracellular trafficking of meprin β, the insertion of a bend at a distinct position in the tail (specifically by a Y685P mutation) suffices to retain this protein in the ER. We propose that the very length of the cytoplasmic tail, as well as its secondary structure are essential for the ER-to-Golgi transport of meprin β, possibly by allowing an interaction with a cargo receptor.

Selected Publications

Schvartz, I., Kreizman, T., Brumfeld, V., Gechtman, Z., Seger, D. and Shmuel Shaltiel, S. (2001) The PKA phosphorylation of vitronectin. effect on conformation and function. Arch. Biochem. Biophys, in press.

Morgenstern, E., Gnad, U., Preissner, K.T., Dierichs, R., Belleli, A., Chestukhin, A., Schvartz, I. and Shaltiel, S. (2001) Localization of protein kinase a and vitronectin in resting platelets and their translocation onto fibrin fibers during clot formation. Eur. J. Cell Biol 80, 87-98.

Seger D., Gechtman, Z. and Shaltiel, S. (1998) Phosphorylation of vitronectin by casein kinase ii. identification of the sites and their promotion of cell adhesion and spreading. J. Biol. Chem., 273, 24805-24813.

Seger, D., Seger, R. and Shaltiel, S. (2001) The CKII phosphorylation of vitronectin. promotion of cell adhesion via the αvβ3 - PI3K pathway. J. Biol. Chem. 276, 16998-17006.

Seger, D., and Shaltiel, S. (2000) Evidence showing that the two-chain form of vitronectin is produced in the liver by a selective furin cleavage. FEBS Letters, 480(2-3) 169-174.

Litovchick, L., Chestukhin, A. and Shaltiel, S. (1998) The C-Terminal Tail of KSMP/Meprin β is Involved in its Intracellular Trafficking. J. Biol. Chem. 273, 29043-29051.

Chestukhin A., Litovchick L., Muradov K., Batkin M. and Shaltiel S. (1997) Unveiling the Substrate Specificity of Meprin b on the basis of the Site in PKA cleaved by the Kinase Splitting Membranal Proteinase. J. Biol. Chem., 272, 3153-3160.

Shaltiel S., Cox S. and Taylor S.S. (1998) Conserved Water Molecules Contribute to the Extensive Network of Interactions at the Active Site of Protein Kinase A Proc. Natl. Acad. Sci. USA, 95, 484-491.

Gechtman, Z., Belleli, A., Lechpammer, S. and Shaltiel, S. (1997) The cluster of basic amino acids in vitronectin contributes to its PAI-1 binding: evidence from thrombin, elastase, and plasmin clipped vitronectins and anti-peptide antibodies. Biochem. J. 325(2), 339-349.

Batkin, M., Schvartz, I. and Shaltiel, S. (2000) Snapping of the carboxyl terminal tail of the catalytic subunit of PKA onto its core: characterization of the sites by mutagenesis. Biochemistry, 39, 5366-5373.

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