

New horizons in vascular biology and thrombosis: Highlights from EMVBM 2009

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The six review articles collected in this Theme Issue of *Thrombosis & Haemostasis* are derived from invited lectures at the 5th European Meeting on Vascular Biology & Medicine (EMVBM) held in September 2009 in Marseille, France and coordinated by Profs. Jose Sampol and Françoise Dignat George. This Theme Issue summarises recent advances in the field of vascular biology and thrombosis that have important implications for translational research and possible clinical applications. By covering a wide spectrum of vascular biology, the EMVBM provides an excellent international platform for the discussion of new horizons in this exciting field. The scientific programme featured more than 36 oral communications, 17 workshops, three plenary sessions, two symposia and more than 200 posters. The programme also featured an inspiring keynote lecture by Bruce Furie on thrombus formation and the role of thiol isomerases. The following overview provides a brief synopsis of proceedings in selected topics covered at the EMVBM 2009.

von Willebrand factor, tissue factor and microparticles in vascular homeostasis

Thrombosis is a critical event for arterial diseases and cardiovascular complications, which together with venous thromboembolic disorders account for considerable

morbidity and mortality (1). Multiple mechanisms prevent the premature initiation of thrombosis and ensure its temporospatial confinement. The multimeric von Willebrand factor (vWF) is a crucial protein in thrombus formation, and its essential functions comprise binding and transportation of the procoagulant factor VIII, mediation of platelet adhesion to reactive surfaces under high shear stress, and subsequent platelet aggregation (2). Lenting et al. summarise regulatory mechanisms that limit interaction of vWF with its relevant counterparts under physiological conditions to avoid spontaneous vascular occlusion through inadequate accumulation of vWF-rich platelet aggregates (3). An intriguing mechanism restraining platelet-rich thrombus formation is the proteolysis of ultra-large vWF at the A2 domain by the ADAMTS13 metalloprotease. Ultra-large vWF multimers secreted by endothelial cells and anchored to their surface are highly prothrombogenic due to their capacity to bind the platelet GPIb-IX-V complex, to spontaneously interact with platelet glycoprotein (GP)Ib α and to enhance shear-induced platelet aggregation. The physiological relevance of ADAMTS13 is illustrated by clinical observations that its deficiency is associated with spontaneous microvascular platelet aggregation and subsequent occlusion of the microvasculature in patients with thrombotic thrombocytopenic purpura. Interestingly, proteolysis of ultra-large vWF is promoted by shear stress, presumably by reversibly stretching ultra-large vWF to an open conformation and exposing the A2 domain. Once cleaved, regular vWF fragments are released from the endothelial surface and adopt a globular shape with limited access for ADAMTS13, rendering resistance to further degradation. vWF activity is also affected by changes in local magnesium concentration, whereas magnesium inhibits ristocetin-induced platelet aggregation and vWF binding to collagen and triggers vWF cleavage. Moreover, pro-

teins present in endothelial Weibel-Palade bodies may interact with vWF and interfere with its platelet-binding capacity. The functionality of the A1 domain may also be modulated by adjacent carbohydrate structures or shear stress-induced conformational changes of vWF resulting in impaired physical accessibility ("shielding") for platelet GPIb α . Overall, deciphering these regulatory pathways opens emerging avenues to understand the role of vWF in clinical situations.

Tissue factor (TF) is the major initiator of the blood coagulation system acting as a cofactor for circulating factor VIIa and inducing a cascade of proteolytic reactions that culminate in the production of thrombin. The essential biologic properties and functions of TF have been elucidated and the concept of vessel wall-derived TF forming a "haemostatic envelope" is well established (4). Owens et al. address the unresolved contribution of circulating TF and the relevance of various haematopoietic cells for the delivery of "blood-borne" TF (5). Genetically altered mice, for instance "low TF" mice or mice with cell-type specific deletions of the TF gene, may help to unravel the involvement of various sources of TF that drive thrombosis. Some animal models of thrombosis indeed indicated that circulating cellular and microparticle-associated TF may provide a mobile source of TF supporting thrombus initiation or expansion under certain conditions. In intact vascular systems it is likely that the concentration of such circulating TF is below a threshold required for thrombus formation or that TF exists in an encrypted low-activity state. Of note, several limitations apply to the use of animal models which warrant caution when drawing definitive conclusions (6). For instance, ferric chloride (FeCl₃)-induced injury can cause collagen-dependent thrombus formation in the microcirculation and FeCl₃ also induces RNA release into the circulation, which may promote thrombus formation. In this context, where TF is not the only pro-

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coagulant stimulus, the relative contribution of circulating TF to thrombus expansion is obviously difficult to evaluate. In general, it appears that sources of TF that drive thrombosis not only depend on the type of blood vessel but also on the animal model. Importantly, genetically altered mice models might be useful to define the role of TF in biological processes besides thrombosis, such as inflammation, angiogenesis and tumour growth. In particular, a link between coagulation, inflammation and tumour growth may be established by TF-signalling activity via protease-activated receptor (PAR)-1 or PAR-2 (7).

A further link between thrombosis, inflammation and angiogenesis is provided by circulating microparticles (MPs). MPs represent a heterogeneous population of vesicles with a diameter of 100–1000 nm, which express antigens specific of their parental cells and are shed during cellular activation or apoptosis. MPs of different cellular origins are found in the plasma of healthy individuals and their circulating levels increase in patients with cardiovascular diseases or malignancies. MPs display a rich spectrum of bioactive substances and receptors on their surface and harbour a concentrated set of cytokines, signalling proteins, mRNA and microRNA. Recent studies provided evidence that MPs may transfer part of their components and content to selected target cells, mediating cell activation, phenotypical modification and reprogramming of cell function (8–10). Readily circulating in the vasculature, MPs may serve as vectors of biological information in their local environment, but also at remarkable distance from their site of origin. Leroyer et al. review mechanisms leading to formation of endothelial microparticles (EMPs), highlight their unique features and discuss their involvement in vascular homeostasis (11). EMPs may constitute both surrogate markers of vascular injury and endothelial dysfunction with prognostic value for major adverse events in patients with cardiovascular diseases and mediators in thrombosis, inflammation, vascular injury and angiogenesis. Of note, EMPs seem to play a Janus-headed role in such complex processes facilitating both primary beneficial and adverse effects. EMPs are not only passive bystanders of endothelial dysfunction, but may actively

induce impairment of vasomotor function. In addition, EMPs may amplify inflammatory reactions, as well as initiate and aggravate thrombotic events via their procoagulatory potential, which is mainly based on the capability to bind different coagulation factors as well as by harbouring and transferring TF. On the other hand, EMPs can support angiogenesis under certain conditions, for instance by promoting matrix degradation via EMP-associated metalloproteinase activity. More recently, EMPs were assigned a new proteolytic function based on their capacity to generate plasmin with potential implication in clot dissolution, tissue remodelling, vascular growth and cancer. The recognition of EMP as vectors of adverse biological effects opens novel perspectives on therapeutic strategies targeting EMPs release.

Effectors of vascular remodelling: stem cells, heme oxygenase and phosphate

Stem cell or progenitor cell-based therapy is an intriguing approach for tissue repair and tissue engineering, which may substantially complement conventional treatment strategies for cardiovascular diseases in the future. Xiao et al. review recent progress in the understanding of the complex processes, environmental cues, and genes that control and mediate differentiation of stem cells into vascular smooth muscle cells (SMCs) (12). SMCs are characterised by their plasticity with the elementary capability to switch their phenotype in response to environmental changes triggered by growth factors, mechanical influences, cell-cell and cell-matrix interactions, and inflammatory mediators. The need for defining and understanding the molecular pathways that promote stem cell differentiation is underlined by evidence that circulating and resident stem/progenitor cell populations can give rise to smooth muscle-like cells during vascular injury, tissue ischemia and atherosclerotic lesion formation and may substantially affect pathophysiological processes in these disease states (13–15). Relevant elements that drive differentiation towards SMCs comprise growth factors like tissue growth factor (TGF)- β and platelet-derived growth factor (PDGF)-BB, interactions

with extracellular matrix components such as collagen IV, and finally activation of integrins (i.e. $\alpha1/\beta1/\alpha4$) and corresponding downstream signal transducers. Moreover, differentiation of SMCs may be dynamically regulated at the level of chromatin through a synergistic interplay of DNA-binding transcription factors, in particular serum response factors (SRF), accessory coactivators for DNA-binding proteins (e.g. myocardin), direct interactions of DNA and transcription factor complexes (e.g. SRF-CArG interaction), and histone modifications present within promoter chromatin (16). Several enzymes could be identified as modulators of SRF, including NADPH oxidase 4, which mediates SRF phosphorylation and translocation via formation of reactive oxygen species and the spliced histone deacetylase 7 known to increase the binding of SRF to SMC gene promoter. Recently, it has been suggested that various microRNAs (e.g. microRNA-145) are directly affected by SRF and target a network of transcription factors to promote SMC reprogramming and differentiation (17). Interestingly, microRNA-145 was found to regulate the phenotype switch of SMCs and is downregulated in injured or atherosclerotic vessels. Together, these new findings may help to develop novel therapeutic strategies for intervention in cardiovascular diseases, e.g. by manipulation or targeted delivery of microRNA.

Regulation and/or promotion of neovascularisation constitutes a highly debated therapeutic approach for treating peripheral and myocardial ischaemia and for controlling tumour growth and wound healing. Grochot-Przeczek et al. provide an in-depth analysis of the role of heme oxygenase-1 (HO-1) in neovascularisation, especially in the context cardiovascular diseases and diabetes (18). The inducible enzyme HO-1 catalyses the rate-limiting step in the oxidative degradation of cellular heme that liberates iron, carbon monoxide (CO), and biliverdin. HO-1 seems to mediate beneficial effects on several pathophysiological states by exerting angiogenic but also anti-inflammatory, anti-apoptotic, and cytoprotective effects (19). It is conceivable that such role of HO-1 evolved as an autoprotective mechanism to respond to several noxious stimuli. Transfection

studies with overexpression of HO-1 as well as siRNA studies suggested that HO-1 stimulates the synthesis and activity of vascular endothelial growth factor (VEGF) and SDF-1 α /CXCL12 in various cell types, establishing a relevant mechanism linking HO-1 to increased blood vessel formation and blood flow in animal models. Furthermore, HO-1 is associated with enhanced mobilisation of endothelial progenitor cells (EPCs) and may promote the vasoregenerative potential of EPCs. An underlying mechanism may be induction of CXCL12 activity triggering CXCR4-related proangiogenic signals in EPCs. Of note, the involvement of HO-1 in neovascularisation appears to be ambivalent and depends on the pathophysiological condition, as angiogenesis driven by lipopolysaccharide-induced inflammation is attenuated, while VEGF-promoted angiogenesis is supported by HO-1 (20). Wound healing and tissue regeneration in HO-1^{-/-} mice were shown to be delayed, whereas adenoviral vector-mediated HO-1 gene transfer accelerated wound healing in diabetic mice, likely supported by the concomitant enhancement of angiogenesis within a wound. Furthermore, HO-1 and generated CO may, at least partially, correct vascular and endothelial dysfunction in animal models of diabetes and may reinforce the lower regenerative potential of EPCs in a hyperglycaemic environment. The data summarised by Grochot-Przeczek et al. suggest that manipulation of the HO-1 gene might be a new avenue in the prevention and/or treatment of cardiovascular diseases and diabetes (19). It remains to be elucidated whether such therapeutic strategies also affect the maturation of nascent blood vessels, a process in particular relevant for inflammatory and VEGF-driven neovascularisation in individuals with atherosclerotic vessels (21).

Chronic kidney disease (CKD) is characterised by phosphate retention and reduced synthesis of 1,25-dihydroxyvitamin D stimulating parathyroid hyperplasia. These mineral and endocrine disturbances cause a complex osteopathy, defined as renal osteodystrophy, which is together with hyperphosphataemia linked to vascular calcification. Such calcifying arteriopathy is associated with arterial stiffness, leads to hy-

per-tension, heart failure and compromised coronary perfusion and contributes to the high cardiovascular mortality and morbidity among CKD patients (22). Lau et al. highlight phosphate-dependent mechanisms entailing vascular calcification with emphasis on the osteochondrogenic phenotype switch of vascular SMCs and the involvement of the type III sodium-dependent phosphate co-transporter PiT-1 (23). Current evidence indicates that increased phosphate uptake by SMCs plays a key role for the reprogramming of vascular SMC lineage to the bone-forming phenotype, which is associated with release of apatite-containing vesicles serving as nucleation sites for calcification. Expression of matrix metalloproteinases is up-regulated in arteries from diabetic CKD patients and may mediate elastin degradation with local deposition of hydroxyapatite resulting in aggravation of arterial stiffness. Although the mechanism responsible for its contribution in vascular calcification has not been fully elucidated, PiT-1 appears to be relevant for mineralisation and osteochondrogenic transition in SMCs. Such an engagement of PiT-1, which may be up-regulated by several calcification-promoting factors, including tumour necrosis factor (TNF)- α , PDGF and elevated calcium relies on increased phosphate uptake. Moreover, a dysbalance between circulating promoters and inhibitors of the calcification process, such as fetuin-A, matrix-Gla protein, osteoprotegerin, bone morphogenetic proteins, and pyrophosphate is critical for the calcification of vascular tissue (24). Despite substantial progress in delineating key players responsible for premature vascular deterioration, the best available tools for preventing and treating calcification in CKD patients remain related to the optimal control of the mineral dysbalance, bone metabolic and inflammatory parameters.

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