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Contribution of proteomics to the management of vascular disorders

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ABSTRACT

Vascular disorders, and in particular atherothrombosis, are currently a leading cause of morbidity and mortality in Western societies. Proteomics research into these disorders has helped improving our knowledge of the underlying mechanisms involved in the development of atherothrombosis, as well as providing novel biomarkers to diagnose and for the prognosis of this disease. However, the application of these advances into clinical use has not followed this trend. In this review we explore the potential of Proteomics and Metabolomics for the management of vascular disorders, paying special attention to atherothrombosis and aiming to guide the reader from the experimental design of proteomic analysis through the initial discovery phase to the clinical implementation of biomarkers or therapeutic targets (Fig. 1), providing state-of-the-art proteomic studies to exemplify the concepts addressed.

1. Atherothrombosis: pathogenesis and clinical needs

Fig. 1 Clinical outcomes of atherothrombosis, including acute coronary syndromes (ACS), stroke and claudication from peripheral artery disease (PAD), represent the most important causes of mortality and morbidity in Western societies. Atherosclerosis originates through endothelial dysfunction, and sub-endothelial LDL (low density lipoprotein) deposition and oxidation, particularly at specific locations of the arterial tree (coronary, aorta, carotid, cerebral, renal and femoral arteries) [1]. Several risk factors predispose individuals to atherogenesis, including dyslipidemia, hypertension, tobacco use, diabetes and obesity. The high prevalence of these factors in the population, together with the increase in life expectancy, account for the overwhelming incidence of atherothrombosis in developed countries. For all these reasons, better clinical management of atherothrombosis will help decrease the death rate from cardiovascular disease (CVD) and improve the quality of life in the population. This goal can be achieved in part by increasing efforts in educational programs that make people aware of the benefits of a healthy lifestyle, namely a healthy diet, avoidance of smoking and regular physical activity [2]. On the other hand, a better understanding of the molecular mechanisms underlying atherothrombosis, and the incorporation of more efficient biomarkers of pathology, would benefit diagnosis, prognosis and may provide novel therapeutic targets, thereby improving clinical management of such patients.

1.1. Cardiovascular risk assessment

The biomarkers widely used to assess the risk of clinical outcomes derived from atherothrombosis are very often used in combination with the Framingham Risk Score (FRS). This score puts traditional cardiovascular risk factors together to calculate the 10-year risk of an adverse cardiovascular outcome, and constitutes the most internationally used predictor of CVD. First defined in 1998 [3], this score owes its name to the Framingham Heart Study, conducted on 2489 men and 2856 women aged 30–74 years old at baseline and over a 12-year follow-up. The FRS is calculated by adding or subtracting points in function of age, systolic blood pressure, LDL-cholesterol, HDL-cholesterol and smoking habit, evaluating the value according to gender. The higher the score, the greater the risk of CVD. The FRS allows populations to be stratified into three categories associated with the probability of developing cardiovascular events in the following 10 years: low (<10%), intermediate (10–20%) and high-risk (>20%). Nevertheless, and despite its great utility in the clinic, the imperfect discriminatory capability of FRS [4,5] requires further refinement of the algorithm in order to improve its value as a CV risk stratification tool.

In terms of molecular biomarkers for CVD, several soluble molecules are currently used to diagnose and predict future outcome, including C-reactive protein (CRP), an inflammatory marker used for CVD risk prediction [6], B-type natriuretic peptides, biomarkers of heart failure (HF) diagnosis [7], and

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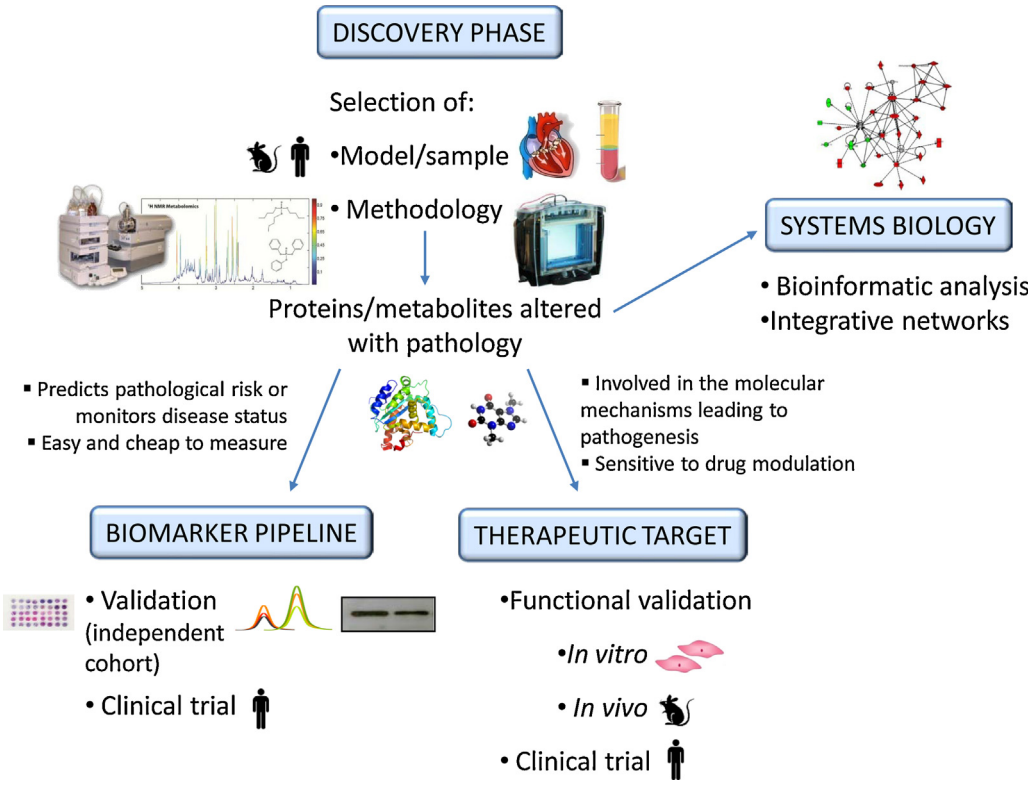

Fig. 1. A schematic view of the flowchart of a comprehensive proteomic/metabolomic analysis in the search for disease biomarkers and therapeutic targets.

cardiac troponins (cTnI, cTnT) to ensure the detection of acute myocardial infarction (AMI) [8] (Fig. 2). Although atherosclerosis is the underlying cause of the majority of cardiovascular events, none of the aforementioned biomarkers are specific biomarkers for the early diagnosis of atherothrombosis.

1.2. Biomarkers of plaque vulnerability

Markers of plaque vulnerability represent a useful tool for clinicians, since unstable plaques are more likely to rupture and provoke thrombosis. The development and vulnerability of an atherome plaque reflects multiple molecular processes associated with lipid accumulation, inflammation, proteolysis, angiogenesis, hypoxia, apoptosis and calcification. Among the biomarkers of plaque vulnerability, lipoprotein-associated phospholipase A2 (Lp-PLA_2) [9,10] and myeloperoxidase (MPO) [11–13] are probably those best demonstrated to be clinically useful. MPO is a heme protein involved in many secondary reactions that generate reactive species and in LDL oxidation, and it contributes to endothelial dysfunction and foam-cell formation [14]. This protein has been proven to predict risk and mortality in ACS patients [11,13], and it constitutes an early biomarker of atherosclerosis, as determined in a prospective study on healthy individuals [12]. Lp-PLA_2 is secreted by inflammatory cells and it binds to circulating LDL, and its deposition is greater in vulnerable plaques [15]. Thus, elevated levels of this protein in plasma have been associated with a higher risk of coronary heart disease [9,10]. Moreover, during atherogenesis, LDLs are deposited in the sub-endothelium and they are oxidized as a result of the pro-oxidative inflammatory milieu therein. Indeed, oxLDL levels are associated with advanced atherosclerosis and they constitute a well-established biomarker for outcome prediction [16].

Plaque rupture is frequently produced by the disruption of the fibrous cap from the atherome plaque, which is mediated by apoptosis of vascular smooth muscle cells (VSMCs) and proteolysis. Matrix metalloproteinases (MMPs) play a crucial role in these events as they degrade the extracellular matrix (ECM). Therefore, blood MMP-9 levels have been associated with cardiovascular risk in a variety of studies [14]. In addition, tissue factor (TF) is a pro-coagulant protein secreted by foam cells and VSMCs during plaque development and it initiates thrombosis after its release with plaque rupture. Elevated blood levels of TF have been associated with unstable angina [17] and increased blood thrombogenicity in type 2 diabetes mellitus [18]. One particularly interesting alternative is to evaluate TF-positive circulating extracellular microvesicles, which exert pro-coagulant ability and constitute a novel biomarker of thrombosis [19,20].

Recently, the fibrotic marker Gal-3 that has proved useful to predict heart failure [21], has been shown to modulate inflammation during the development of atherosclerosis [22], and its blood levels are associated with unstable angina [23] and increased cardiovascular mortality [24]. Moreover, several inflammatory biomarkers that are associated with plaque vulnerability have been shown to be useful to predict cardiovascular outcomes (sCD40L, IL-6, IL-18, MCP-1, etc.), although such results should be "handled with care", since underlying inflammatory pathologies may account for the observed changes in these biomarkers. Conversely, adhesion molecules that are over-expressed in endothelial dysfunction (VCAM-1, ICAM-1) are widely used as biomarkers of vascular function in the follow-up of high-risk cardiovascular patients [25].

Despite of the availability of these biomarkers, which are crucial for the diagnosis and risk assessment of CVD, more research focusing on the molecular mechanisms driving atherothrombosis, and on the identification of earlier, more discriminating and more specific biomarkers of the disease is still needed. In this sense, panels of biomarkers may be of great utility for diagnostic and prognostic purposes, providing better sensitivity and specificity. Indeed, the discrimination of particular patient sub-groups expressing specific panels of these biomarkers would increase