

Proteomics of Acute Coronary Syndromes

Athanasios Didangelos, BSc, PhD, David Simper, MD, Claudia Monaco, MD, PhD, and Manuel Mayr, MD, PhD

Corresponding author

Manuel Mayr, MD, PhD

Cardiovascular Division, The James Black Centre, King's College London School of Medicine, King's College London, 125 Coldharbour Lane, London SE5 9NU, United Kingdom.

E-mail: manuel.mayr@kcl.ac.uk

Current Atherosclerosis Reports 2009, 11:188–195

Current Medicine Group LLC ISSN 1523-3804

Copyright © 2009 by Current Medicine Group LLC

Acute coronary syndromes (ACS), such as unstable angina, acute myocardial infarction, and sudden cardiac death, are commonly associated with the presence of vulnerable plaques in coronary arteries. Rupture or erosion of vulnerable plaques results in the formation of luminal thrombi due to the physical contact between platelets and thrombogenic elements within the atherosclerotic lesions. Considering the socioeconomic burden of ACS, it is imperative that the scientific community achieves a clear understanding of the multifaceted pathophysiology of vulnerable atheroma to identify accurate prognostic biomarkers and therapeutic targets. The analytical power of modern proteomic technologies could facilitate our understanding of vulnerable plaques and lead to the discovery of novel therapeutic targets and diagnostic biomarkers.

Introduction

The pathologic features of atherosclerotic plaques define their propensity to rupture. Vulnerable atherosclerotic plaques are generally characterized by the presence of a necrotic, foam-cell lipid core separated from the arterial lumen by a thin and disrupted fibrous cap that exhibits a clear reduction in collagen content. The fibrous cap is heavily infiltrated by activated macrophages and T lymphocytes [1]. In contrast, histologic examination of stable plaques shows significantly reduced macrophage infiltration and a thicker fibrous cap with a clearly increased population of smooth muscle cells (SMCs) [2]. It is important to note that approximately one third of acute luminal thrombi arise from eroded plaques. Compared

with ruptured plaques, erosions demonstrate smaller necrotic cores and a proteoglycan-rich fibrous cap with more SMCs and fewer macrophages. The thrombus is usually formed in direct contact with the intima in an area of absent endothelium [3]. The fibrous cap is largely characterized by its dense extracellular matrix (ECM), a complex network of structural molecules such as fibrillar and nonfibrillar collagens and proteoglycans. The quantity and quality of the ECM in the fibrous cap are of paramount importance in defining plaque stability. Notably, SMCs residing in the fibrous cap are responsible for the synthesis of these macromolecules and for the dynamic ECM remodelling. Hence, it is not surprising that the presence of SMCs in the fibrous cap is believed to stabilize the plaque and prevent it from rupturing via the production and maintenance of ECM [4]. However, the presence of macrophages and T lymphocytes is considered to be deleterious because they are responsible for the production of inflammatory mediators and proteolytic enzymes.

Current Understanding of the Vulnerable Plaque: Beyond Inflammation

Contemporary atherosclerosis research has primarily focused on the inflammatory component of the disease. Excellent studies based on the analysis of human plaques and cell culture studies have shown the presence of activated immune cells (mainly macrophages, T lymphocytes, and mast cells) at the sites of plaque rupture [5]. Their biological activity appears to induce dysregulation of ECM metabolism and, consequently, plaque destabilization. For example, activated T cells secrete interferon- γ , a potent inhibitor of collagen synthesis by SMCs [6], and CD40 ligand, an activator of matrix metalloproteinases (MMPs) in SMCs [7]. Similarly, macrophages secrete proinflammatory cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor- α (TNF- α), which amplify the inflammatory response and upregulate MMP gene expression by SMCs [8]. Activated macrophages also promote SMC death [9••]. Most importantly, however, macrophages produce a wide range of MMPs [10] that have the ability to degrade most ECM components, including collagens I

and III and proteoglycan core proteins [11••], resulting in plaque destabilization.

The importance of inflammation on atherosclerosis is supported by findings in murine models. Hypercholesterolemic apolipoprotein E (apoE) or low-density lipoprotein receptor knockout mice have often been crossed with mice bearing genetic deletion of elements of innate and adaptive immunity, such as Toll-like receptors (TLR2/4), cytokines and their receptors (IL-1/IL-1R, TNF- α /TNF- α R), and chemokines (CCL2/CCR2). These studies have shown that inflammatory mediators are involved in the pathophysiology and progression of the disease in vivo. However, they have failed to address the molecular events leading to plaque destabilization and rupture [9••]. The main limitation of the current approaches is their focus on the investigation of individual factors, which are presumed to be involved in the pathophysiology of plaque rupture and whose biological functions are, at least in part, understood. Given the biological complexity of the vulnerable plaque, research into atherosclerosis will benefit from a more holistic approach aiming at a comprehensive analysis of the microenvironment within lesions.

Proteomics: A Promising Armamentarium for Research on Atherosclerosis

The complexity of atherosclerotic plaques results from the heterogeneous cell composition and the different proteins secreted by local cells and inflammatory cells infiltrating the tissue. The promise of proteomic techniques is to offer an unbiased platform for the comprehensive analysis of proteins rather than investigating individual molecules. Although mRNA expression is widely considered to be indicative of protein expression, the actual protein levels within the lesion are the net result of protein synthesis and degradation. Although mRNA levels may correlate with protein synthesis, they are not informative of protein degradation. In contrast, the proteomic approach investigates protein expression directly. This is particularly important in the context of atherosclerosis, in which protein degradation is a key determinant of actual protein concentrations. Moreover, proteomics reveals posttranslational modifications (eg, phosphorylation, oxidation, glycation, nitrosylation, and so forth), which are known to be instrumental for many human diseases. Undoubtedly, large-scale studies of protein expression could facilitate the discovery of novel proteins, biomarkers, or mechanisms of disease [12].

Proteomic methodologies: choice matters

Modern proteomic methodologies and their application to atherosclerosis research were described in detail in a review published recently in this journal [13]. Thus, this article only highlights some of the key differences

in the methodologies currently employed for proteomic analysis. There are two key strategies in contemporary proteomic research.

Separation at the protein level by two-dimensional gel electrophoresis

Two-dimensional gel electrophoresis (2DE) offers powerful separation of proteins by utilizing two principal physical properties of proteins: charge and molecular mass. In the first dimension, proteins are separated according to their isoelectric point within an immobilized pH gradient, a process called isoelectric focusing (IEF). In the second dimension, sodium dodecyl sulfate polyacrylamide gel electrophoresis is used to further separate the proteins according to their molecular mass. Importantly, 2DE not only allows the separation of proteins from complex biological samples, but it also enables the visualization of posttranslational modifications or isoforms of the same protein as a shift in their isoelectric point or molecular mass [14]. The current gold standard in 2DE is difference in gel electrophoresis (DIGE) [15]. DIGE is performed by labelling different protein samples with distinct fluorescent probes. The different samples can then be co-separated in the same 2DE gel and co-detected by using the overlay of their fluorescent patterns. An internal standard labelled with a third dye is used for normalization. The DIGE technology is exceptionally useful for quantitative comparisons because it minimizes gel-to-gel variations and substantially improves the accuracy of the quantitative analysis compared with other techniques, such as silver staining. Following separation by 2DE, proteins of interest are excised from the gel and digested by trypsin. The tryptic peptides are then subjected to analysis by mass spectrometry, and there are two major approaches.

The first, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, involves determining the masses of the intact peptides, which produces a “peptide mass fingerprint” of a sample. MALDI-TOF, however, is not considered state of the art for reliable protein identification because it does not provide direct evidence for the amino acid sequence of a protein. The second approach, tandem mass spectrometers (MS/MS), not only records the mass of the peptide ions but also induces a random fragmentation process. The masses of these fragment ions can be used to verify the amino acid sequence of the peptide. In this way, the protein sequence can be established and used to search sequence databases to find related and exact matches. For analysis, peptides are usually separated by reverse-phase, high-pressure liquid chromatography to which the tandem mass spectrometer is coupled in-line (LC-MS/MS). Modern tandem mass spectrometers offer attomole on-column sensitivity and very high mass accuracy. In addition, novel methods for the fragmentation of ionized peptides, such as electron transfer dissociation, enable the preservation

of posttranslational modifications such as phosphorylation or glycosylation, which are routinely lost during collision-induced dissociation, which is currently the most commonly used fragmentation method.

Separation at the peptide level by gel-free “shotgun” proteomics

In “shotgun” proteomics, protein mixtures are subjected to enzymatic digestion followed by chromatographic separation and analysis by tandem mass spectrometry. Quantification relies on differential labelling of peptides with stable isotopes. Commonly used methods are stable isotope labelling with amino acids in cell culture (SILAC) or isobaric tags for relative and absolute quantification (iTRAQ). For SILAC, the culture medium is supplemented with amino acids tagged with nonradioactive isotopes (ie, ^{14}C). The corresponding peptides containing the light and heavy isotopes can be readily differentiated by their mass difference. This technique can reliably detect quantitative differences; however, complete labelling with stable isotopes in culture is only achieved over five population doublings. By this time, most primary cells are considered too old for further analysis. For iTRAQ, proteins are not a priori labelled in culture, but an isobaric mass tag is introduced to unlabelled proteins after digestion with trypsin. Upon fragmentation, this isobaric mass tag generates low molecular mass reporter ions that differ in size. There are currently four-plex and eight-plex reagents that allow relative quantification of proteins originating from four or eight different samples in a single analysis, respectively. The disadvantage of any shotgun approach is that the number of different samples/treatments that can be analyzed is limited and that the quantification is readily compromised by experimental variation (ie, incomplete labelling or digestion of the analytes). Moreover, shotgun proteomics does not quantify actual protein level but relies on peptides to estimate protein abundance. Reliable quantification is more readily obtained for high abundant components for which multiple peptides are detected and accurately quantified. For low abundant proteins, reliable quantification can be impossible, especially in complex biological samples. Thus, although the gel-free approach is more sensitive in identifying low abundant proteins, it is not necessarily better for quantifying proteins in very complex biological samples or tissues.

Proteomics of atherosclerosis

Few studies have used proteomic approaches to investigate atherosclerotic lesions in human or animal tissue. In one of the first attempts to compare protein expression in atherosclerotic versus normal coronary arteries by 2DE, You et al. [16] reported elevated expression of ferritin light chain in diseased arteries. The authors concluded that ferritin light chain might contribute to

coronary atherosclerosis by modulating the oxidation of lipids in the vessel wall [16]. In a more recent study, laser capture microdissection was used to obtain samples from very specific areas of human coronary atherosclerotic tissue sections [17]. Proteomic analysis of these samples was then carried out by a gel-free LC-MS approach that generated 495 protein identifications with multiple peptides. Although laser capture microdissection enabled the analysis of protein content from very specific areas of atherosclerotic lesions, quantitative comparisons between diseased and normal tissues remain a challenge, as differences in cellular composition will be largely responsible for the observed changes in protein content. To overcome this limitation, we have performed a proteomic and metabolomic analysis of aortas obtained from apoE knockout mice. We were particularly interested in the different stages of the disease process and tried to identify alterations of the proteome and metabolome before the onset of atherosclerosis. Our findings revealed that inefficient glucose and energy metabolism coincide with increased oxidative stress in aortas of apoE^{-/-} mice even before lesion development. This was evidenced by oxidation of redox-sensitive proteins, such as peroxiredoxin 6, in aortas of young apoE^{-/-} mice. Moreover, attenuation of lesion formation was associated with replenishment of the vascular energy pool and posttranslational modifications of cytosolic malic enzyme, which provides reducing equivalents for lipid synthesis and glutathione recycling [14]. In an alternative approach, Martin-Ventura et al. [18] employed 2DE and mass spectrometry to compare the secretome of cultured carotid atherosclerotic endarterectomy specimens with normal arterial specimens. The most significant finding was a reduction of heat shock protein 27 (hsp27) in the culture supernatant of the atherosclerotic plaques. The clinical relevance of this finding was further substantiated by reduced plasma levels of hsp27 in a small cohort of patients undergoing carotid endarterectomy [18]. Interestingly, a very recent 2DE proteomic analysis of stable versus unstable carotid plaque extracts confirmed a significant reduction in stress response proteins, including hsp27, hsp20, superoxide dismutase 3, and others, in unstable plaques [19]. Larger studies, however, are needed to establish whether small heat shock proteins can be of clinical value as biomarkers for vascular disease.

Proteomics of plasma

No study has directly applied proteomics to atherosclerotic plaques from coronary arteries. Mateos-Caceres et al. [20] reported an analysis of modifications in the plasma proteome during unstable angina and acute myocardial infarction. Using 2DE and mass spectrometry, they identified significant changes in four major plasma proteins: α 1-antitrypsin, apoA-1, fibrinogen, and immunoglobulin γ (heavy chain) [20]. In another study, proteomic analysis of serum taken from patients suffering from acute myocardial

Table 1. Findings revealed by proteomic analysis applied to atherosclerosis and ACS

Study / year	Tissue	Proteomic techniques	Findings
You et al. [16] / 2003	Normal vs atherosclerotic human coronary artery (tissue extracts)	2DE and LC-MS/MS	Elevated ferritin light chain in the atherosclerotic coronary artery extract
Bagnato et al. [17] / 2007	Normal and atherosclerotic human coronary artery (sections)	Specific areas of tissue sections were excised by laser capture microdissection; proteins were separated by chromatographic fractionation and identified by LC-MS	495 protein identifications
Martin-Ventura et al. [18] / 2004	Normal radial or mammary artery vs atherosclerotic carotid plaque (secretome)	2DE and LC-MS/MS	Reduction of hsp27 protein expression in the carotid plaque supernatant
Lepedda et al. [19] / 2008	Stable vs unstable carotid plaque (tissue extracts)	2DE and LC-MS/MS	Reduction of hsp27, hsp20, and SOD3 protein expression in the unstable plaque extracts
Mayr et al. [14] / 2005	Wild-type vs APOE ^{-/-} aortas, light vs severe lesions (tissue extracts)	2DE and LC-MS/MS; NMR spectroscopy	Impaired energy metabolism and increased oxidative stress during atherogenesis
Mateos-Caceres et al. [20] / 2004	Normal plasma vs unstable angina and AMI patient plasma	2DE and LC-MS/MS	Changes in plasma from patients with AMI: AAT (decreased), fibrinogen gamma chain (increased), APOA-1 (decreased), and IgG heavy chains (increased)
Barderas et al. [22] / 2007	Monocytes isolated from either stable patients or non-STEMI ACS patients	2DE and LC-MS/MS	18 proteins were significantly altered and 10 were absent in the non-STEMI ACS monocytes
Eberini et al. [21] / 2007	Serum from AMI patients	2DE and LC-MS/MS	APOA-1 degradation products in the serum of patients with AMI

2DE—two-dimensional gel electrophoresis; AAT—alpha-antitrypsin; ACS—acute coronary syndrome; AMI—acute myocardial infarction; APO—apolipoprotein; hsp—heat shock protein; LC—liquid chromatography; MS—mass spectrometer; MS/MS—tandem mass spectrometers; NMR—nuclear magnetic resonance; SOD3—superoxide dismutase 3; STEMI—ST-segment elevation myocardial infarction.

infarction revealed significant degradation of apoA-1 by utilizing the visualization of APOA-1 cleavage products on 2DE [21]. The authors concluded that apoA-1 degradation was at least in part the result of *in vivo* proteolytic activity by MMPs. Of course, many more changes in the plasma proteome would be expected, including the release of established markers for myocardial damage, such as troponin I and C, creatine kinase, and lactate dehydrogenase. It is also noteworthy that plasma proteomics failed to identify C-reactive protein, an emerging biomarker of plaque vulnerability. However, the dynamic range of protein concentrations in plasma spans nine orders of magnitude, whereas current proteomic technologies can only resolve four orders of magnitude. 2DE, in particular, will be restricted to the most abundant plasma proteins, limiting its potential use for biomarker discovery. Similarly, in “shotgun” proteomics, the vast number of peptides originating from the most abundant plasma proteins (ie, albumin and immunoglobulins) will mask

the less abundant peptides derived from cytokines or growth factors or proteins leaking from diseased tissues. More recently, Barderas et al. [22] analyzed the proteome of cultured monocytes taken from patients suffering from ACS with non-ST-segment elevation. Upon admission, 18 proteins (eg, cathepsin D, HSP60, HSP70, and S100A8) were found to be significantly altered in the proteome of the ACS group when compared with the proteome of monocytes taken from stable patients. Interestingly, the proteomic pattern of the ACS monocytes changed over time, and 6 months after the acute event it resembled the proteomic profile of monocytes from stable patients. The main findings of proteomic studies published to date are summarized in Table 1. Similar to proteomics, novel metabolomic technologies allow the sensitive analysis of multiple metabolites from cells or body fluids. The use of metabolomics for the analysis of novel elements involved in cardiovascular pathology is emerging. A fine example is a recent study by Lewis et al. [23]. The authors used

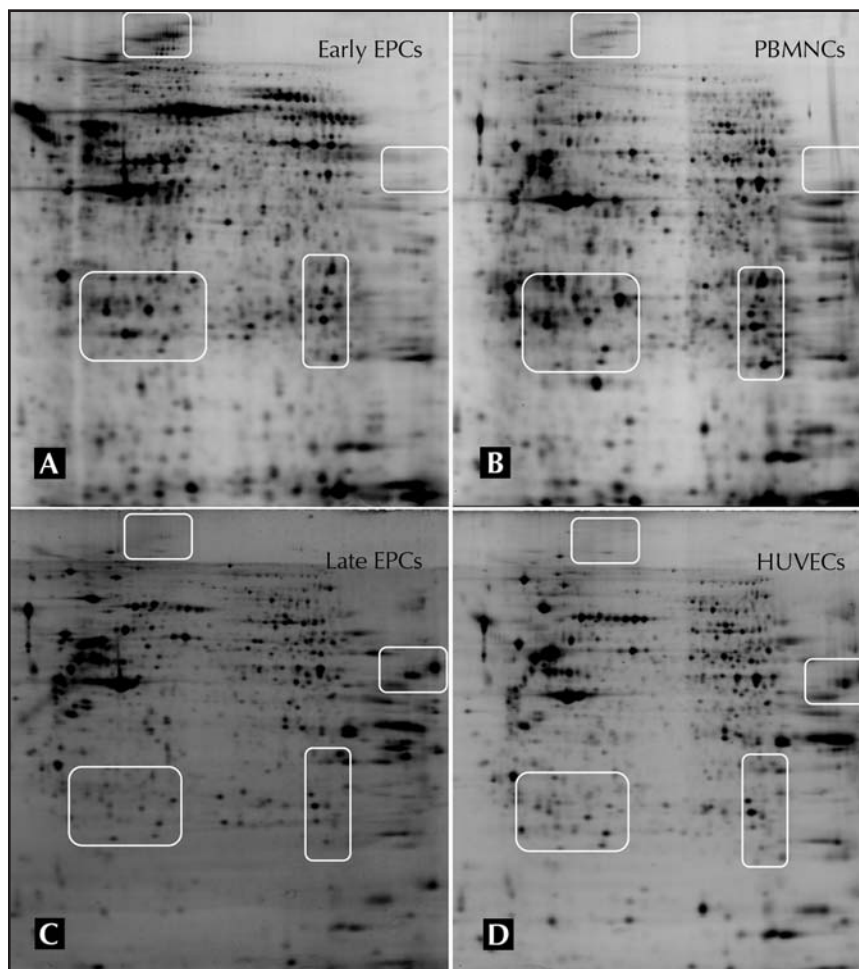


Figure 1. Proteomic profiling to phenotype endothelial progenitor cell (EPC) populations. HUVEC—human umbilical vein endothelial cell; PBMNC—peripheral blood mononuclear cell.

LC-MS to analyze early changes in the plasma metabolite profile from patients with planned myocardial infarction. Interestingly, they found novel changes in plasma levels of pyrimidine metabolites, such as orotic acid, malonic acid, and aminoisobutyric acid. Such changes in the metabolomic profile could be used for the very early detection of myocardial infarction [23].

Proteomics on vascular progenitors

Recent evidence suggests that endothelial progenitor cells (EPCs) and smooth muscle progenitor cells (SPCs) are present in bone marrow and circulate in peripheral blood [24•]. EPCs are probably the most widely studied adult progenitor cell type, but several issues regarding their characterization and activity remain unclear. Notably, despite a decade of research, there is still no specific marker for their unambiguous identification, and small clinical trials using EPCs as a cell-based therapy following myocardial infarction produced controversial results [25]. Microarray analysis provided important insights into the possible paracrine effects of EPCs by demonstrating that EPCs secrete high levels of cathepsins [26]. This finding was further substantiated by proteomics, which revealed the presence of additional angiogenic

factors [27]. Interestingly, the increased proteolytic activity of EPCs would explain why infusion of EPCs in apoE^{-/-} mice increased plaque size and decreased markers indicative of plaque stability [28]. Proteomics could enhance the characterization of such cell populations (an example is shown in Figure 1). In contrast, recent data on SPCs suggest a more benevolent nature of this cell type. For instance, Zoll et al. [29••] demonstrated that intravenous injection of human SPCs in double knockout apoE/rag2 mice decreased plaque development and promoted a stable plaque phenotype. In contrast, injection of EPCs had no effect. In addition to circulating progenitors, resident stem cells in the vasculature have attracted considerable attention. For example, we have recently demonstrated that stem cell antigen 1–positive progenitors exist in the vascular adventitia of apoE^{-/-} mice [30]. These resident progenitors migrate from the adventitia to the media during atherosclerotic lesion development. Using 2DE and mass spectrometry, we carefully characterized this cell population and showed that they acquire characteristics of mature SMCs upon incubation with platelet-derived growth factor. Moreover, the phenotype of progenitor-derived SMCs more closely resembled apoE^{-/-} rather than wild-type SMCs, and our proteomic

data demonstrate the advantage of using an unbiased proteomic approach to assess cellular phenotypes [31].

Proteomics on extracellular matrix

As mentioned previously, the two main determinants of plaque stability are the ECM of the fibrous cap and the presence of SMCs, which are believed to stabilize the plaque and prevent it from rupture. The ECM composition of the fibrous cap has been studied mostly through electron microscopy visualization and histochemical and immunohistochemical staining of a few specific ECM molecules in atherectomy specimens [32]. The most prominent components of the fibrous cap are fibrillar collagens, small elastin fibers, and proteoglycans. Other minor elements, such as nonfibrillar collagens, have also been detected in the fibrous cap [33].

Fibrillar collagens type I and III comprise approximately 60% of total protein in the fibrous cap [34]. Type I is largely responsible for tensile strength, whereas type III confers elasticity. In contrast to the normal tunica intima, which contains more type III collagen, the fibrous cap is particularly rich in type I, indicating decreased resilience and susceptibility to hemodynamic forces [33]. Nevertheless, the presence of fibrillar collagen in the fibrous cap is generally associated with stability. In contrast, sites of plaque rupture are characterized by a marked reduction in fibrillar collagen and increased collagen proteolysis. The potential role of nonfibrillar collagens in the fibrous cap, or in atherogenesis in general, remains unknown. Type VIII collagen has recently received more attention. It has been shown to accumulate in atherosclerotic lesions and may play a role in plaque stabilization [35].

Proteoglycans are important macromolecules of the fibrous cap [36]. Together with type I collagen, they comprise the bulk of ECM synthesized by the SMCs in the fibrous cap. Their structure is characterized by a protein core, which is substituted by heavily sulfated glycosaminoglycan (GAG) chains. The number of GAG chains attached to the protein core is very variable, as is the sulfation pattern of GAGs. These negatively charged structures attract water and confer swelling pressure to the collagen network. An important feature of proteoglycans is that their core protein associates with the large nonsulfated glycosaminoglycan hyaluronic acid to form essential ECM aggregates. The proteoglycans that have been shown to reside in the fibrous cap are versican (substituted exclusively with chondroitin sulphate GAG chains), biglycan and decorin (substituted with dermatan sulphate GAG chains), and perlecan (substituted with heparan sulphate GAG chains).

Very little is known about the role of proteoglycans in vulnerable atherosclerotic lesions. Historically, chondroitin sulfate-rich and dermatan-sulfate rich proteoglycans and hyaluronic acid have been shown to associate strongly with the proatherogenic apoB-containing lipoprotein

particles (ie, low-density lipoprotein and very low-density lipoprotein) to form highly aggregated complexes [37]. The presence of these particles is strongly related to atherogenesis, and accumulation of proteoglycans is believed to increase plaque burden [38]. Other studies have shown that in advanced atherosclerotic lesions, the proteoglycan-hyaluronic acid aggregates are smaller and less stable, indicating a possible role in the decreased integrity of the ECM [39]. An interesting study by Kolodgie et al. [40] showed that at sites of plaque rupture there was severe reduction of versican, biglycan, and hyaluronic acid, together with reduction in type I collagen, indicating extensive ECM degradation. Interestingly, the presence of decorin was unaffected [40]. However, apart from immunostaining, no comprehensive analysis of the extracellular matrix has been performed.

A comparative and unbiased proteomic analysis focused on the ECM components of stable versus unstable atherosclerotic plaques could identify new connective tissue molecules potentially involved in the formation of atherosclerotic lesions. The presence of proteoglycans, nonfibrillar collagens, and other molecules needs to be assessed in the context of plaque vulnerability. Furthermore, it is also important to investigate whether structural alterations of connective tissue molecules, especially proteolytic processing, participate in the pathology of the vulnerable plaque. For instance, it has been shown that hyaluronic acid degradation products (eg, tetra- and hexasaccharides) can act as endogenous TLR-2 and TLR-4 ligands and induce a proinflammatory response in a variety of cell types, including macrophages and endothelial cells [41]. Currently, the pathologic consequences of depolymerized hyaluronic acid are being studied extensively in relation to chronic lung diseases, osteoarthritis, and rheumatoid arthritis. Similarly, the fibronectin splice variant extra type III domain A has been shown to activate T cells and induce MMP-9 expression in human monocytes by activating TLR-4 [42], but no such studies have been conducted in unstable atherosclerotic lesions.

Proteomics on proteolytic products of the ECM

The proteolytic activity of MMPs is a key regulator of ECM integrity. MMPs are a family of 23 proteinases in humans that play a key role in ECM turnover under normal conditions. They are also involved in the increased ECM breakdown observed during disease processes such as atherosclerosis. Several studies have shown increased expression and activity of MMPs, including MMP-1, -2, -3, and -9, in vulnerable areas of atherosclerotic plaques [43]. Other studies have shown increased collagenolysis in vulnerable plaques. Collagenolysis was detected by antibodies raised against type I collagen neoepitopes and was attributed to the proteolytic activities of MMP-1 and -13 [44]. Interestingly, synthesis of active MMP-9

by macrophages and SMCs was demonstrated in human coronary atherectomy specimens taken from patients with unstable angina but not from stable patients [45]. Unfortunately, recent efforts to inhibit MMP activity using synthetic MMP inhibitors in an attempt to improve clinical outcome have been inconclusive [46]. This may be due to the absence of selective MMP inhibitors or due to the complexity of in vivo proteolytic regulation. Indeed, in vivo proteolytic regulation involves the activity of the natural MMP inhibitors, which are the tissue inhibitors of metalloproteinases (TIMPs). TIMP-1, -2, and -3 collectively inhibit all metalloproteinases with different substrate specificity. The regulation of their activity in the context of plaque vulnerability is poorly understood, and one could speculate that the fine balance between MMP-TIMP activity is responsible for plaque stability in some individuals or vulnerability in others. Moreover, MMP transgenic mice have also produced inconsistent results regarding atherogenesis. A number of cathepsins, which are cysteine proteases that can degrade collagen and elastin, have been identified in macrophages in the shoulder region of advanced atheroma [47]. Whether cathepsins participate in the destabilization of vulnerable plaques remains to be determined. Finally, little is known regarding the regulation of proteoglycan degradation. Halpert et al. [48] found that versican is a substrate for MMP-7 and MMP-12 at sites of plaque rupture. A class of metalloproteinases, namely the ADAMTSs (a disintegrin and metalloproteinase with thrombospondin motifs), have recently received attention because they induce very potent and specific proteolysis of the aggrecan core protein. Jonsson-Rylander et al. [49] demonstrated that ADAMTS-1 can degrade versican, but other functions of ADAMTSs in atherosclerosis are still unclear. Proteomics could advance our understanding on the regulation of ECM catabolism. For instance, proteomic analysis of tissue extracts could result in the identification of a wide range of proteinases and inhibitors present in the vulnerable plaque. More importantly, proteomics could identify specific ECM degradation products that could be associated with the activity of specific proteinases in atherosclerotic lesions.

Conclusions

The protein expression profile of cells and tissues defines their biological properties. Instead of investigating only a limited and preselected number of proteins, proteomics enables us to analyze global protein expression. In the context of ACS, proteomic analysis of vulnerable plaques can provide novel information regarding the pathologic mechanisms of plaque rupture. The unbiased dataset generated by proteomic techniques may reveal novel biomarkers and therapeutic targets that can be exploited for clinical practice.

Acknowledgments

This work was funded by grants from the British Heart Foundation. Dr. Mayr is supported by a Senior Research Fellowship of the British Heart Foundation.

Dr. Didangelos is affiliated with the Cardiovascular Division at King's College London in the United Kingdom.

Dr. Simper is affiliated with the Department of Cardiology at the Carl T. Hayden Veterans Affairs Medical Center in Phoenix, AZ and the Harrington Department of Biotechnology at Arizona State University in Tempe, AZ.

Dr. Monaco is affiliated with the Kennedy Institute of Rheumatology at Imperial College London in the United Kingdom.

Disclosure

No potential conflicts of interest relevant to this article were reported.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- Of importance
 - Of major importance
1. Libby P: Molecular bases of the acute coronary syndromes. *Circulation* 1995, 91:2844–2850.
 2. Kolodgie FD, Virmani R, Burke AP, et al.: Pathologic assessment of the vulnerable human coronary plaque. *Heart* 2004, 90:1385–1391.
 3. Virmani R, Burke AP, Farb A, Kolodgie FD: Pathology of the vulnerable plaque. *J Am Coll Cardiol* 2006, 47:C13–18.
 4. Libby P: Current concepts of the pathogenesis of the acute coronary syndromes. *Circulation* 2001, 104:365–372.
 5. van der Wal AC, Becker AE, van der Loos CM, Das PK: Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. *Circulation* 1994, 89:36–44.
 6. Amento EP, Ehsani N, Palmer H, Libby P: Cytokines and growth factors positively and negatively regulate interstitial collagen gene expression in human vascular smooth muscle cells. *Arterioscler Thromb* 1991, 11:1223–1230.
 7. Schonbeck U, Mach F, Sukhova GK, et al.: Regulation of matrix metalloproteinase expression in human vascular smooth muscle cells by T lymphocytes: a role for CD40 signaling in plaque rupture? *Circ Res* 1997, 81:448–454.
 8. Loppnow H, Werdan K, Buerke M: Vascular cells contribute to atherosclerosis by cytokine- and innate-immunity-related inflammatory mechanisms. *Innate Immun* 2008, 14:63–87.
 - 9.•• Yan ZQ, Hansson GK: Innate immunity, macrophage activation, and atherosclerosis. *Immunol Rev* 2007, 219:187–203.
- This is a review on the inflammatory component of atherosclerosis and plaque instability.
10. Galis ZS, Sukhova GK, Lark MW, Libby P: Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. *J Clin Invest* 1994, 94:2493–2503.
 - 11.•• Nagase H, Visse R, Murphy G: Structure and function of matrix metalloproteinases and TIMPs. *Cardiovasc Res* 2006, 69:562–573.

This is a brief and clear review on MMPs and TIMPs.

12. Mayr M, Mayr U, Chung YL, et al.: Vascular proteomics: linking proteomic and metabolomic changes. *Proteomics* 2004, 4:3751–3761.
 13. Martinez-Pinna R, Martin-Ventura JL, Mas S, et al.: Proteomics in atherosclerosis. *Curr Atheroscler Rep* 2008, 10:209–215.
 14. Mayr M, Chung YL, Mayr U, et al.: Proteomic and metabolomic analyses of atherosclerotic vessels from apolipoprotein E-deficient mice reveal alterations in inflammation, oxidative stress, and energy metabolism. *Arterioscler Thromb Vasc Biol* 2005, 25:2135–2142.
 15. Unlu M, Morgan ME, Minden JS: Difference gel electrophoresis: a single gel method for detecting changes in protein extracts. *Electrophoresis* 1997, 18:2071–2077.
 16. You SA, Archacki SR, Angheloiu G, et al.: Proteomic approach to coronary atherosclerosis shows ferritin light chain as a significant marker: evidence consistent with iron hypothesis in atherosclerosis. *Physiol Genomics* 2003, 13:25–30.
 17. Bagnato C, Thumar J, Mayya V, et al.: Proteomics analysis of human coronary atherosclerotic plaque: a feasibility study of direct tissue proteomics by liquid chromatography and tandem mass spectrometry. *Mol Cell Proteomics* 2007, 6:1088–1102.
 18. Martin-Ventura JL, Duran MC, Blanco-Colio LM, et al.: Identification by a differential proteomic approach of heat shock protein 27 as a potential marker of atherosclerosis. *Circulation* 2004, 110:2216–2219.
 19. Lepedda AJ, Cigliano A, Cherchi GM, et al.: A proteomic approach to differentiate histologically classified stable and unstable plaques from human carotid arteries. *Atherosclerosis* 2008 Jul 12 (Epub ahead of print).
 20. Mateos-Caceres PJ, Garcia-Mendez A, Lopez Farre A, et al.: Proteomic analysis of plasma from patients during an acute coronary syndrome. *J Am Coll Cardiol* 2004, 44:1578–1583.
 21. Eberini I, Gianazza E, Breggi L, et al.: Apolipoprotein A-I breakdown is induced by thrombolysis in coronary patients. *Ann Med* 2007, 39:306–311.
 22. Barderas MG, Tunon J, Darde VM, et al.: Circulating human monocytes in the acute coronary syndrome express a characteristic proteomic profile. *J Proteome Res* 2007, 6:876–886.
 23. Lewis GD, Wei R, Liu E, et al.: Metabolite profiling of blood from individuals undergoing planned myocardial infarction reveals early markers of myocardial injury. *J Clin Invest* 2008, 118:3503–3512.
 24. Simper D, Stalboerger PG, Panetta CJ, et al.: Smooth muscle progenitor cells in human blood. *Circulation* 2002, 106:1199–1204.
- This article reviews the controversy regarding EPCs and SPCs.
25. Hristov M, Zernecke A, Schober A, Weber C: Adult progenitor cells in vascular remodeling during atherosclerosis. *Biol Chem* 2008, 389:837–844.
 26. Urbich C, Heeschen C, Aicher A, et al.: Cathepsin L is required for endothelial progenitor cell-induced neovascularization. *Nat Med* 2005, 11:206–213.
 27. Pula G, Mayr U, Evans C, et al.: Proteomics identifies thymidine phosphorylase as a key regulator of the angiogenic potential of colony-forming units and endothelial progenitor cell cultures. *Circ Res* 2009, 104:32–40.
 28. George J, Afek A, Abashidze A, et al.: Transfer of endothelial progenitor and bone marrow cells influences atherosclerotic plaque size and composition in apolipoprotein E knockout mice. *Arterioscler Thromb Vasc Biol* 2005, 25:2636–2641.
 29. Zoll J, Fontaine V, Gourdy P, et al.: Role of human smooth muscle cell progenitors in atherosclerotic plaque development and composition. *Cardiovasc Res* 2008, 77:471–480.
- This article addresses the controversy regarding EPCs in the context of atherosclerosis.
30. Hu Y, Zhang Z, Torsney E, et al.: Abundant progenitor cells in the adventitia contribute to atherosclerosis of vein grafts in ApoE-deficient mice. *J Clin Invest* 2004, 113:1258–1265.
 31. Mayr M, Zampetaki A, Sidibe A, et al.: Proteomic and metabolomic analysis of smooth muscle cells derived from the arterial media and adventitial progenitors of apolipoprotein E-deficient mice. *Circ Res* 2008, 102:1046–1056.
 32. Ross R, Wight TN, Strandness E, Thiele B: Human atherosclerosis. I. Cell constitution and characteristics of advanced lesions of the superficial femoral artery. *Am J Pathol* 1984, 114:79–93.
 33. Plenz GA, Deng MC, Robenek H, Volker W: Vascular collagens: spotlight on the role of type VIII collagen in atherogenesis. *Atherosclerosis* 2003, 166:1–11.
 34. Rauterberg J, Jaeger E, Althaus M: Collagens in atherosclerotic vessel wall lesions. *Curr Top Pathol* 1993, 87:163–192.
 35. Plenz G, Dorszewski A, Volker W, et al.: Cholesterol-induced changes of type VIII collagen expression and distribution in carotid arteries of rabbit. *Arterioscler Thromb Vasc Biol* 1999, 19:2395–2404.
 36. Wagner WD: Proteoglycan structure and function as related to atherosclerosis. *Ann N Y Acad Sci* 1985, 454:52–68.
 37. Wight TN: Proteoglycans in pathological conditions: atherosclerosis. *Fed Proc* 1985, 44:381–385.
 38. O'Brien KD, Olin KL, Alpers CE, et al.: Comparison of apolipoprotein and proteoglycan deposits in human coronary atherosclerotic plaques: colocalization of biglycan with apolipoproteins. *Circulation* 1998, 98:519–527.
 39. Wagner WD, Edwards IJ, St Clair RW, Barakat H: Low density lipoprotein interaction with artery derived proteoglycan: the influence of LDL particle size and the relationship to atherosclerosis susceptibility. *Atherosclerosis* 1989, 75:49–59.
 40. Kolodgie FD, Burke AP, Farb A, et al.: Differential accumulation of proteoglycans and hyaluronan in culprit lesions: insights into plaque erosion. *Arterioscler Thromb Vasc Biol* 2002, 22:1642–1648.
 41. Scheibner KA, Lutz MA, Boodoo S, et al.: Hyaluronan fragments act as an endogenous danger signal by engaging TLR2. *J Immunol* 2006, 177:1272–1281.
 42. Okamura Y, Watari M, Jerud ES, et al.: The extra domain A of fibronectin activates Toll-like receptor 4. *J Biol Chem* 2001, 276:10229–10233.
 43. Dollery CM, Libby P: Atherosclerosis and proteinase activation. *Cardiovasc Res* 2006, 69:625–635.
 44. Sukhova GK, Schonbeck U, Rabkin E, et al.: Evidence for increased collagenolysis by interstitial collagenases-1 and -3 in vulnerable human atheromatous plaques. *Circulation* 1999, 99:2503–2509.
 45. Brown DL, Hibbs MS, Kearney M, et al.: Identification of 92-kD gelatinase in human coronary atherosclerotic lesions. Association of active enzyme synthesis with unstable angina. *Circulation* 1995, 91:2125–2131.
 46. Galis ZS, Khatir JJ: Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. *Circ Res* 2002, 90:251–262.
 47. Sukhova GK, Shi GP, Simon DI, et al.: Expression of the elastolytic cathepsins S and K in human atheroma and regulation of their production in smooth muscle cells. *J Clin Invest* 1998, 102:576–583.
 48. Halpert I, Sires UI, Roby JD, et al.: Matrilysin is expressed by lipid-laden macrophages at sites of potential rupture in atherosclerotic lesions and localizes to areas of versican deposition, a proteoglycan substrate for the enzyme. *Proc Natl Acad Sci U S A* 1996, 93:9748–9753.
 49. Jonsson-Rylander AC, Nilsson T, Fritzsche-Danielson R, et al.: Role of ADAMTS-1 in atherosclerosis: remodeling of carotid artery, immunohistochemistry, and proteolysis of versican. *Arterioscler Thromb Vasc Biol* 2005, 25:180–185.