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REVIEW

Proteomics in antiphospholipid syndrome: a review

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The presence of antiphospholipid antibodies (aPL) has been closely related to the development of thrombosis and complications in pregnancy. However, not all patients with aPL will develop those clinical features. The exact pathogenic mechanisms leading to thrombosis and/or pregnancy morbidity are poorly understood. Currently, biomarkers which enable one to predict the prognosis of patients with positive aPL are not readily available. Current advances in genomics and proteomics provide the opportunity to discover novel biomarkers based on changes in concentration levels or post-translational modifications of proteins and peptides. These techniques are now being applied in various areas of medicine with very promising results. This review covers recent studies that have used this approach for a better understanding of the pathogenic mechanisms involved in the development of thrombosis in patients with antiphospholipid syndrome (APS). Although, there are very few qualified biomarkers that have arisen as a result of efforts in proteomics, it is expected that these techniques will deliver biomarkers that might ultimately identify different subgroups of APS patients with various prognoses that might have implications with respect to management and prognosis. *Lupus* (2010) **19,** 385–388.

Key words: Antiphospholipid syndrome; thrombosis; intracellular signals

Introduction

Antiphospholipid syndrome (APS) is a disorder characterized by the association of arterial or venous thrombosis and/or pregnancy morbidity, with the presence of antiphospholipid antibodies (aPL): anticardiolipin antibodies (aCL) and/or lupus anticoagulant (LA) and/or anti-β_2-glycoprotein I antibodies (anti-β_2GPI).[1]

Different mechanisms have been proposed to explain the role of aPL in the development of thrombosis in APS patients but, to date, the precise pathogenesis of the prothrombotic state associated with aPL remains unknown.

Genomic and proteomic studies have proven quite useful in the identification of pathogenic mechanisms in a large variety of disorders. Gene analysis can be completed by studying the much larger number of proteins encoded by them. Several proteins can be generated from a single gene depending on how the genetic information is read (transcribed) and how the resultant protein is modified following translation (post-translational modification).[2] Therefore, analysis of messenger RNA (mRNA) expression alone is insufficient to determine whether the proteins encoded are actually synthesized. Proteomics may be able to compensate for these genomic limitations.

Both genomic and proteomic technologies have already been applied for a better understanding of the pathogenic mechanisms in different autoimmune diseases.[3,4] From the genetic point of view, it is known that there is a complex interaction between the product of various genes, and genomic analyses can tell us which genes are altered in different tissues from patients with autoimmune diseases. Recent genomic and transcriptomic profiling studies have implicated certain cytokines, surface receptors, signalling pathways, and cell types in the pathogenesis of inflammatory diseases.[5]

The proteomic pattern of peripheral blood mononuclear cells (PBMCs) has been described in systemic lupus erythematosus (SLE)[6] and other autoimmune diseases.[7] These studies are providing solid data about gene and protein expression deregulation that may help to identify different diseases and the subset of patients who are at risk of more severe disease.

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The pathogenic mechanisms leading to thrombosis in APS patients remain unclear. One of the most promising findings includes the induction of tissue factor (TF) expression by endothelial cells and monocytes.[8–10] The intracellular mechanisms underlying the aPL-induced TF gene and protein expression in endothelial cells and monocytes have also been described. aPL antibodies induce TF expression in monocytes from APS patients by activating, simultaneously and independently, the phosphorylation of mitogen-activated protein kinase 1/extracellular signal regulated kinase (MEK1/ERK) proteins, and the p38 mitogen-activated protein (MAP) kinase-dependent nuclear translocation and activation of nuclear factor-κB (NF-κB/Rel proteins).[11,12]

Genetic factors have been also investigated, but the heterogeneity in antigen specificity and in the pathophysiology of thrombosis make it difficult to determine genetic risk factors associated with the development of APS. Many candidate genes, including HLA class II haplotype, have shown a predisposition towards APS.[13] A recent genomic study was carried out in PBMCs of aPL positive patients in order to search for patterns of gene expression that can predict the risk for venous thrombosis in APS patients.[14] The authors were able to describe gene-expression patterns from patients' peripheral blood that may predict an individual's predisposition towards developing thrombosis. Some of the genes identified in this study included those encoding apolipoprotein E (ApoE), factor X and thromboxane, all of them linked to the pathophysiology of thrombosis. In addition, other genes were identified in patients with APS that have thus far not been directly linked to venous thrombosis, including those encoding for hypoxia inducible factor (HIF-1alpha), zinc finger proteins, matrix metalloproteinase 19 (MMP19), interleukin 22 (IL22) receptor, and hematopoietic progenitor cell antigen (CD34) precursor, among others.

Our group has also addressed the question of predicting thrombotic risk in APS patients by using a proteomic approach on purified human monocytes.[15] Four groups of patients were included in this study: APS patients with thrombosis, APS patients only with pregnancy morbidity, who had never had a thrombotic event, patients with thrombosis but without APS and healthy donors. The proteins identified as more significantly deregulated in the monocytes from patients with APS and thrombosis, when compared with the other three groups, were annexin A1 (A1), annexin A2 (A2), ubiquitin Nedd8, Rho A protein, protein disulfide isomerase (PDI) and Hsp60. These proteins have been shown to be associated with the induction of a procoagulant state, as well as autoimmune-related responses.

Two annexins were found to be upregulated in monocytes of APS samples: A1 and A2. Annexins are a family of phospholipid- and calcium-binding proteins that have been implicated in many cellular processes, including immune responses. A1 is expressed by many different tissues and its levels are also elevated in other autoimmune diseases, such as multiple sclerosis, experimental autoimmune encephalomyelitis and rheumatoid arthritis.[16,17] A recent study has shown that increasing the expression of A1 leads to the constitutive activation of ERK1/2 in RAW macrophages.[18] Accordingly, we have found that the upregulation of AI in APS monocytic cells was accompanied by the constitutive activation of the MEK/ERK pathway (unpublished data).

A2, is a receptor for fibrinolytic activation localized on the cell surface of endothelial cells, monocytes and syncytiotrophoblasts.[19] A2 has recently been directly involved in the pathogenesis of APS. It has been demonstrated that binding of β_2GPI to human umbilical vein endothelial cells is mediated by A2.[20] Studies have demonstrated that β_2GPI binds to A2 on the surface of endothelial cells.[20] Furthermore, thrombosis and TF upregulation are significantly decreased in A2 deficient mice in vivo. In that study an anti-A2 antibody ameliorated in vitro and in vivo affects aPL antibodies.[21] By functioning as a receptor for β_2GPI, A2 is a target not only for anti-A2 antibodies but also for anti-β_2GPI antibodies, which are direct inductors of TF. These data suggest that A2 might constitute a common receptor for aPL induction of monocyte activation.[22]

In our studies, ubiquitin-like protein Nedd8 was significantly increased in monocytes of APS patients. This protein is involved in the proteolytic destruction of IκB (inhibitor of NF-κB), which allows nuclear translocation of free NF-κB, thus leading to activation of a multitude of target genes.[23] In monocytes and endothelial cells from APS, a constitutive activation of NF-κB has been described, which was further related to an aPL-induced TF expression found in these patients.[6,24]

APS patients with thrombosis also showed a significant increase in the expression of Rho A proteins when compared with the remaining

analysed groups. Rho A proteins play critical roles in inflammatory signal transduction cascades, such as those required for the activity of NF-κB.[25] In addition, it has been demonstrated that inhibition of Rho/Rho kinase proteins downregulates the synthesis of TF by cultured human monocytes, and that statins suppress the synthesis of TF by inhibition of Rho activity.[26] Rho A proteins may be directly involved in monocyte APS activation.

Protein disulphide isomerase (PDI) is associated with TF on the cell surface when coagulant activity is low and TF-VIIa signalling is enabled. PDI expression reduction was associated with a two-fold increase of TF procoagulant activity.[27] It has been demonstrated that overexpression of PDI suppresses NF-κB-dependent transcriptional activity.[28] As the aberrant activation of the NF-κB signalling pathway is likely to contribute to the development of APS, the decrease expression of this protein might be related to the constitutive activation of this transcription factor in the APS.

Finally, the heat shock protein 60 (Hsp60) was found downregulated in monocytes from APS patients: Hsp60 is present in the blood during inflammation, and has been found to be a target of autoantibodies and autoimmune T-cells in healthy individuals, as well as those suffering from autoimmune diseases.[29]

The identification of two proteins (fibrinogen and haemoglobin) that might be related to the aetiology of recurrent pregnancy loss of the APS patients should also be mentioned.[15] The expression levels of fibrinogen in APS without thrombosis was found to be lower than in the other study groups. Recent studies have suggested that the absence of – or a significant decrease in – maternal fibrinogen is sufficient to cause rupture of vasculature, affecting embryonic trophoblast infiltration and leading to haemorrhagic miscarriage.[30] Deficiencies of fibrinogen during gestation may lead to abnormal growth of foetus or abortion. Similarly, a recent study has demonstrated the increased gene expression of haemoglobin in patients with recurrent spontaneous abortions.[31] The altered expression of these proteins might be helpful in understanding the molecular mechanisms underlying pregnancy morbidity in APS patients. However, these results have to be taken with caution since only nine patients with APS and obstetric complications were included in this study.

Table 1 summarizes some of the genomic and proteomic markers of thrombotic risk in APS described to date.

Summary

The application of proteomic techniques to APS patients' monocytes has led one to identify an altered expression of proteins. This abnormal expression might be directly related to the pathogenic mechanisms of APS.

Although some results have still to be confirmed in larger studies, the use of proteomic biomarkers might be a useful tool to identify different

Table 1 Genomic and proteomic markers of thrombotic risk in antiphospholipid syndrome (APS). Some examples of genes are given. See cited references for a complete list of genes and proteins identified

Genes/proteins associated with thrombosis in APS			*Technique utilized*	*Change*	*References*
Gene	*Protein*	*Accession*			
Pattern of 50 genes expressed in PBMCs that predict thrombotic risk in APS patients			DNA microarrays		Potti *et al.*[14]
PTGIR	Prostacyclin receptor	D38127		Down	
MMP19	Matrix Metalloproteinase-19	U89651		Down	
F10	Coagulation factor X	L00390		Down	
HSPCA	Heat Shock Protein-90	M27024		Down	
APOE	Apolipoprotein E	M10065		Down	
PDIA6	Protein Disulfide Isomerase a6	Q15084		Down	
REQ	Zinc-finger Protein UBI-D4	U43920		Down	
HIF1A	Hypoxia-inducible Factor 1 α	AF050127		Down	
IL22RA1	Interleukin 22 Receptor	Q8N6P7		Down	
Proteins differentially expressed in blood monocytes from APS with thrombosis patients			2DE-MALDI TOF		López-Pedrera *et al.*[15]
	Annexin 1	P04083		Up	
	Annexin 2	P07355		Up	
	Ubiquitin-like protein nedd8	Q15843		Up	
	Transforming protein RhoA	P61586		Up	
	Protein disulfide isomerase	P07237		Down	

subgroups of APS patients with different prognoses and might lead to different therapeutic decisions.

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References

1 Miyakis S, Lockshin, MD, Atsumi T, *et al*. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost* 2006; 4: 295–306.
2 Peter JF, Otto AM, Wolf B. Magnetic particles as powerful purification tool for high sensitive mass spectrometric screening procedures. *Proteomic* 2009; epub ahead of print.
3 Chan SM, Utz PJ. The challenge of analyzing the proteome in humans with autoimmune diseases. *Ann N Y Acad Sci* 2005; 1062: 61–68.
4 Dotzlaw H, Eggert M, Neeck G, Schulz M. Spots, blots, peaks and chips: proteomic approaches in autoimmune diseases. *Curr Pharm Des* 2006; 12: 3699–3706.
5 Gibson D, Banha J, Penque D, *et al*. Diagnostic and prognostic biomarker discovery strategies for autoimmune disorders. *J Proteomic* 2009; epub ahead of print.
6 Dai Y, Hu C, Huang Y, Huang H, Liu J, Ly T. A proteomic study of peripheral blood mononuclear cells in systemic lupus erythematosus. *Lupus* 2008; 17: 799–804.
7 Li QZ, Zhou J, Lian Y. Interferon signature gene expression is correlated with autoantibody profiles in patients with incomplete lupus syndromes. *Clin Exp Immunol* 2010; 159: 281–291.
8 Cuadrado MJ, López-Pedrera Ch, Khamashta MA, *et al*. Thrombosis in primary antiphospholipid syndrome. A pivotal role for monocyte tissue factor expression. *Arthritis Rheum* 1997; 40: 834–841.
9 Amengual O, Atsumi T, Khamashta MA, Hughes GRV. The role of the tissue factor pathway in the hypercoagulable state in patients with the antiphospholipid syndrome. *Thromb Haemost* 1998; 79: 276–281.
10 Zhou H, Wolberg AS, Roubey RAS. Characterization of monocyte tissue factor activity induced by IgG antiphospholipid antibodies and inhibition by dilazep. *Blood* 2004; 104: 2353–2358.
11 Vega-Ostertag M, Casper K, Swerlick R, Ferrara D, Harris EN, Pierangeli SS. Involvement of p38 MAPK in the up-regulation of tissue factor on endothelial cells by antiphospholipid antibodies. *Arthritis Rheum* 2005; 52: 1545–1554.
12 López-Pedrera Ch, Buendía P, Cuadrado MJ, *et al*. Antiphospholipid antibodies from antiphospholipid syndrome patients induce monocyte expression through the simultaneous activation of both NFκB/Rel proteins via p38 MAPK pathway, and the MEK1/ERK pathway. *Arthritis Rheum* 2006; 54: 301–311.
13 Sebastiani GD, Galeazzi M, Tincani A, *et al*. HLA-DPB1 alleles association of anticardiolipin and anti-beta2GPI antibodies in a large series of European patients with systemic lupus erythematosus. *Lupus* 2003; 12: 560–563.
14 Potti A, Bild A, Dressman HK, *et al*. Gene-expression patterns predict phenotypes of immune-mediated thrombosis. *Blood* 2006; 107: 1301–06.
15 López-Pedrera C, Cuadrado MJ, Hernández V, *et al*. Proteomic analysis in monocytes of antiphospholipid syndrome patients. Deregulation of proteins related to the development of thrombosis. *Arthritis Rheum* 2008; 58: 2835–44.
16 Probst-Cousin S, Kowolik D, Kuchelmeister K, Kayser C, Neundorfer B, Heuss D. Expression of annexin-1 in multiple sclerosis plaques. *Neuropathol Appl Neurobiol* 2002; 28: 292–300.
17 Goulding NJ, Dixey J, Morand EF, *et al*. Differential distribution of annexins-I, -II, -IV, and –VI in synovium. *Ann Rheum Dis* 1995; 54: 841–845.
18 Alldridge LC, Harris HJ, Plevin R, Hannon R, Bryant CE. Regulation of the mitogen activated protein kinase/ERK pathway by the annexin lipocortin 1. *J Biol Chem* 1999; 274: 37620–37628.
19 Falcone DJ, Borth W, Faisal Khan KM, Hajjar KA. Plasminogen-mediated matrix invasion and degradation by macrophages is dependent on surface expression of annexin II. *Blood* 2001; 97: 777–784.
20 Cesarman-Maus G, Rios-Luna NP, Deora AB, *et al*. Antibodies against the fibrinolytic receptor, annexin 2, in antiphospholipid syndrome. *Blood* 2006; 107: 4375–4382.
21 Romay-Penabad Z, Montiel-Manzano MG, Shilagard T, *et al*. Annexin A2 is involved in antiphospholipid antibody mediated pathogenic effects in vitro and in vivo. *Blood* 2009; 114: 3074–3083.
22 Zhang J, McCrae KR. Annexin A2 mediates endothelial cell activation by antiphospholipid/anti-β2 glycoprotein I antibodies. *Blood* 2005; 105: 1964–1969.
23 Dunoyer-Geindre S, de Moerloose P, Galve-de Rochemonteix B, Reber G, Kruithof EK. NFkappaB is an essential intermediate in the activation of endothelial cells by anti-beta (2)-glycoprotein 1 antibodies. *Thromb Haemost* 2002; 88: 851–857.
24 Raschi E, Testoni C, Bosisio D, *et al*. Role of the MyD88 transduction signalling pathway in endothelial cell activation by antiphospholipid antibodies. *Blood* 2003; 101: 3495–3500.
25 Rolfe BE, Worth NF, World CJ, Campbell JH, Campbell GR. Rho and vascular disease. *Atherosclerosis* 2005; 183: 1–16.
26 Nagata K, Ishibashi T, Sakamoto T, *et al*. Rho/Rho-kinase is involved in the synthesis of tissue factor in human monocytes. *Atherosclerosis* 2002; 163: 39–47.
27 Ahamed J, Versteeg HH, Kerver M, *et al*. Disulfide isomerization switches TF from coagulation to cell signalling. *Proc Natl Acad Sci U S A* 2006; 103: 13932–13937.
28 Higuchi T, Watanabe Y, Waga I. Protein disulfide isomerase suppresses the transcriptional activity of NFkB. *Biochem Biophys Res Commun* 2004; 318: 46–52.
29 Wallin RPA, Lundquist A, More SH, von Bonin A, Kiessling R, Ljunggren H-G. Heat shock proteins as activators of the innate immune system. *Trends Immunol* 2002; 23: 130–135.
30 Kim YS, Kim MS, Lee SH, *et al*. Proteomic analysis of recurrent spontaneous abortion: identification of an inadequately expressed set of proteins in human follicular fluid. *Proteomics* 2006; 6: 3445–3454.
31 Baek KH. Aberrant gene expression associated with recurrent pregnancy loss. *Mol Hum Reprod* 2004; 10: 291–297.