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REVIEW

Biomarker Profiling for Lupus Nephritis

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Abstract Lupus nephritis (LN) is one of the most severe manifestations of systemic lupus erythematosus (SLE), which is associated with significant morbidity and mortality of SLE patients. The pathogenesis of LN involves multiple factors, including genetic predisposition, epigenetic regulation and environmental interaction. Over the last decade, omics-based techniques have been extensively utilized for biomarker screening and a wide variety of variations which are associated with SLE and LN have been identified at the levels of genomics, transcriptomics and proteomics. These studies and discoveries have expanded our understanding of the molecular basis of the disease and are important for identification of potential therapeutic targets for disease prediction and early treatment. In this review, we summarize some of the recent studies targeted at the identification of LN-associated biomarkers using genomics and proteomic approaches.

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by production of autoantibodies against a broad range of self-antigens including DNA, RNA, histones and other nuclear components. In most patients, vital organs and tissues are often implicated, including kidney, brain, cardiovascular, joint and skin. Lupus nephritis (LN) is a common and serious complication in SLE and is associated with significant mortality and morbidity of SLE patients. Generally, 74% of lupus patients will develop clinically relevant nephritis at some time in the course of their illness [1]. The pathogenesis of LN is a complex process, involving deposition of autoantibodies in the glomerulus, activation of complement and macrophages, cell proliferation, production of extracellular matrix proteins, pro-inflammatory cytokines and chemokines, which are then linked through multiple mechanisms to cause tubular damage, tubulointerstitial inflammation and fibrosis. So far, LN remains a major challenge and continues to be one of the most severe manifestations of SLE [2]. The medical therapy for LN depends on the severity of the disease. Thus, finding reliable biomarkers for LN will help to evaluate disease activity, identify patients at risk for kidney damage and facilitate early diagnosis and intervention to improve favorable outcomes [3].

Multiple lines of evidence have supported a genetic etiology in SLE and LN. Linkage analysis and Genome-wide association studies (GWAS) have been used for screening lupus susceptibility and analyses of common genetic variants in lupus have revealed a number of susceptibility loci [4,5]. Nonetheless,

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the cumulative effect of these loci accounts for only a fraction of disease heritability [6,7]. Other heritable factors of complex human disease may broadly reside in epigenetic mechanisms. Epigenetics refers to heritable modifications that regulate gene expression without causing DNA sequence changes. Common epigenetic mechanisms include DNA methylation, histone modification and regulation by microRNAs (miRNAs). The variations at the genetic and epigenetic level may be reflected by the different level of protein biomarkers such as autoantibodies and cytokines in serum and urine of LN patients. In this paper, we will briefly review recent discoveries of genetic and proteomic biomarkers, which are associated with susceptibility and pathogenesis of LN.

Genes and genetic variations associated with LN

SLE susceptibility is a complex trait. Some linkage studies have assessed many candidate genes for potential roles in predisposing to SLE. A comparative study of relative risk of disease in siblings of patients with that of the larger population indicated that SLE has a strong genetic basis [8]. In addition, genetic analyses of inbred murine models of systemic autoimmunity also strongly support a genetic predisposition for SLE susceptibility [9]. Further studies have shown that the genetic basis of LN predisposition exhibits in two aspects. On one hand, some susceptibility alleles of candidate genes are associated with LN disease severity. On the other hand, there exists a set of kidney-specific genes that are likely to amplify or sensitize patients to autoimmune pathology of LN [10].

Advances in high-throughput genotyping technology and completion of the Human Genome Project facilitate the development of genome-wide searches for genetic polymorphisms to SLE and LN. The availability of the entire genome sequence has drastically changed the study of genetic predisposition of LN during the past several years. Chromosomal regions containing possible susceptibility loci can be identified by linkage analysis based on the genetic location. Until now, many candidate genes of LN predisposition have been identified.

Histocompatibility complex (MHC) has been demonstrated to be linked to lupus traits, nephritis and production of autoantibodies [11]. Freedman et al. reported human leukocyte antigen (HLA)-B8 and DR2 are positively associated with LN in patients of both African Americans (blacks) and whites [12]. HLA-DQA and HLA-DR alleles have also been shown to be associated with LN in an Italian population in another study [4]. Furthermore, several studies have shown that low-affinity variants of both Fcγ receptor (FcγR) IIA and IIIA are associated with SLE and LN, and FcγRIIA genes are important disease susceptibility factors for SLE, particularly for LN, suggesting that the Fc receptor may influence clinical manifestation of LN [13,14]. In addition, copy number variation of the rat and human Fcgr3 genes is a determinant of susceptibility to immunologically mediated glomerulonephritis, which provides direct evidence for the importance of genome plasticity in the evolution of genetically complex phenotypes [15]. A meta-analysis on the V158F variant of FcγRIIIA revealed an increased risk of LN for individuals with two copies of the risk allele (F/F) [16]. The FcgRIIIA-V/F158 polymorphism may be a susceptibility factor for SLE and LN, play an important role in the pathogenesis of the disease and show prognostic and therapeutic implications as well [16,17]. The predominant distribution of FcγRIIA homozygous R/R131 genotype observed in LN patients indicated that this genotype is a heritable risk factor for immune complex mediated renal injury in Brazilian lupus patients [18].

Genome-wide transcriptomic analysis has uncovered differential gene expression profile in murine LN. By microarray analysis of glomerular gene expression, Teramoto et al. identified 567 up-regulated genes in the glomeruli of MRL/lpr mice with LN compared to control congenic mice. Those included complement components, adhesion molecules, chemokines and their receptors, and molecules related to antigen presentation [19]. Allam et al. observed a greater expression and activation of mouse double minute 2 homolog (Mdm2) gene in the spleen and kidneys in a mouse model of lupus (MRL-Fas^{lpr} mice) than healthy controls. Their data suggested that the induction of Mdm2 promotes the expansion of plasma cells, which cause autoantibody production and immune complex disease in MRL-Fas^{lpr} mice [20]. Therefore, antagonizing Mdm2 may have therapeutic potential in LN. Liu et al. identified kallikrein (KLK) genes that are associated with the susceptibility to anti-glomerular basement membrane (anti-GBM) antibody-induced nephritis and LN [21]. The polymorphisms in the promoter region of KLK genes are responsible for the differential expression of KLK-kinin in the kidney of different mice strains, and the higher expression of KLK plays a protective role against LN [22,23]. Another study on lupus mouse model reported that STAT4-deficient NZM mice developed accelerated nephritis and displayed increased mortality in the absence of high levels of anti-double-stranded DNA antibodies (anti-dsDNA Abs) and in the presence of relatively reduced levels of IFN-γ. In contrast, STAT6-deficient NZM mice exhibited a significant reduction in incidence of kidney disease with a dramatic increase in survival, in the presence of high levels of anti-dsDNA Abs. These studies indicate that STAT4 may be associated with LN [7].

In recent years, more candidate genes have been identified. For example, the programmed cell death 1 (PDCD1 or PD1) PD1.3A allele was reported to be a risk factor for LN in European descendants [6]. PDCD1 gene variation was also associated with LN and these findings confirmed PDCD1 as a LN susceptibility marker [24]. In addition, Jonsen et al. reported that variation in the promoter of the pentraxin C-reactive protein (CRP) gene has been associated with SLE or SLE nephritis in Caucasian and African ethnicities [25]. Among the LN susceptible genes, angiotensin-converting enzyme (ACE) and angiotensinogen (AGT) are the best illustrated renal-specific factors. Two polymorphisms, Alu insertion/deletion (I/D) and 23 949 $(CT)_{2/3}$, in the ACE gene that were correlated with serum ACE levels have now been associated with LN in several studies [26,27], while the M235T polymorphism in the AGT gene was also shown to be associated with LN in Asians [26]. Furthermore, meta-analysis showed the ACE D allele or DD genotype could be a predictive marker for risk of SLE or LN. D allele and DD homozygous are significant genetic markers to predict SLE susceptibility, while DD genotype is a valuable marker to predict the LN risk [28].

Functional genetic polymorphisms of some cytokines and chemokines have been identified to be correlated with LN. For example, –2518 A/G polymorphism of inflammatory mediator monocyte chemoattractant protein-1 (MCP-1) has been shown to be associated with LN [29]. The low frequencies of the risk alleles of the integrin alpha M (ITGAM) gene in

Table 1 Candidate genes associated with LN

Gene	Full name	Variation	Ref
Kidney-specific targeting			
FCGR3A	Fc γ receptor III-A	V/F158	[14]
FCGR3B	Fc γ receptor III-B	Copy number variation (CNV)	[15]
ACE	Angiotensin converting enzyme	Alu I/D	[27]
MCP-1	Monocyte chemoattractant protein-1	A–2518G	[29]
AGT	Angiotensinogen	M235T	[26]
IL-8	Interleukin-8	T–845C	[31]
PAI-1	Plasminogen activator inhibitor-1	–675 4G4G indel	[32]
eNOS	Endothelial nitric oxide synthase	Intron 4 repeat	[33]
EPCR	Endothelial protein C receptor	A6936G	[34]
Amplification of the autoimmune pathology			
CCR5	C-C chemokine receptor 5	D32	[35]
SPP1	Osteopontin	C707T	[36]
HLA-DQA	DQ alpha	DQA*0101	[4,12]
HLA-DQB	DQ beta	DQB*0201	[4,12]
PDCD1	Programmed cell death 1	PD1.3G/A	[24]
ER	Estrogen receptor	PpXx	[37]
MBL2	Mannose binding lectin 2	Gly54Asp	[38]
UG	Uteroglobin	A38G	[39]
IFNG	IFNγ	Allele 114	[40]

Asian SLE populations confirmed that it is a risk factor related to disease susceptibility and probably severe manifestations of SLE [30]. Association studies evaluate candidate genes based on their function in the immune system or their aberrant expression in lupus patients [10]. Some important candidate genes associated with LN are summarized in **Table 1**.

Aberrations in epigenetic regulation for LN

Immune-mediated LN is multigenic and/or multifactorial in origin. Epigenetic mechanisms get involved in a variety of autoimmune disorders, including LN, by regulating immunogenicity and autoantibody production. The influence of epigenetic mechanisms on LN has been investigated in many studies. Epigenetic modifications can influence gene expression and alter cellular function without modifying the genomic sequence. Three main epigenetic processes include DNA methylation, nucleosome repositioning by histone modifications and miRNAs [41].

DNA methylation

Until now, DNA methylation is the most widely studied epigenetic modification. Usually, methylation of cytosine in the regulatory sequences of DNA is associated with transcriptional inactivation of genes, whereas hypomethylation is related to the activation of transcription. Many studies have indicated correlation of DNA methylation with the pathogenesis of SLE. Hypomethylated state of genes in T and B lymphocytes has been generally observed in SLE patients [41,42]. For instance, the lymphocytes from SLE patients displayed gene hypomethylation when compared to normal controls. Studies using demethylating agents indicate that DNA hypomethylation plays a pathophysiological role in SLE and LN. The hypomethylated state and demethylated DNA fragments in the serum of SLE patients can induce the production of anti-DNA antibodies, which are involved in the pathophysiology of SLE and LN [41,43].

The degree of hypomethylation was thought to be correlated with lupus disease activity. Numerous methylation-sensitive genes are over-expressed in lupus $CD4^+$ T cells. Also, $CD8^+$ T lymphocyte and NK cell specific perforin (PRF1) was over-expressed due to hypomethylation modification of DNA in SLE patients [44] and overexpression of serine/threonine protein phosphatase gene PP2A was reported in SLE patients [45]. Wen et al. reported hypomethylation was crucial for apoptotic DNA to induce SLE-like autoimmune disease in SLE-non-susceptible mice by producing high levels of anti-dsDNA Abs, proteinuria and glomerulonephritis [46]. Reduced CD5 expression caused by hypomethylation of an intracellularly expressed truncated CD5 variant (CD5-E1B) on the surface of B lymphocytes promotes auto-reactivity in SLE [47]. The involvement of DNA methylation in X chromosome provides a potential explanation for the female predominance in SLE [47]. In $CD4^+$ T cells of female SLE patients, demethylation with 5-azacytidine results in overexpression of the CD40 ligand, which is a B lymphocyte co-stimulatory molecule encoded on the X chromosome. However, hypomethylation is not associated with SLE for male SLE patients due to the fact that the male X chromosome is demethylated under physiological conditions [48].

Histone modifications

The histone octamer consists of two copies of each of the histone proteins H2A, H2B, H3 and H4. Histone complexes possess flexible N terminal tails that are accessible to posttranslational modifications and can strongly impact the functional capacities of nucleosomes [41]. Histone modification patterns in SLE are complicated. Firstly, systemic inflammation activates a systemic immune response, and subsequently causes apoptosis or death of lymphocytes, which drives the release of nuclear particles. Nucleosomes released from apoptotic cells cannot be cleared sufficiently, which leads to the accumulation of excessive amount of chromatin debris (DNA, histones and nucleosomes) in the circulation and tissues [49]. Autoreactive B cells can further be activated by taking up the released nuclear particles and differentiate into

autoantibody-producing plasma cells, which drives polyclonal immune complex formation and causes glomerulonephritis in the kidney [49]. During LN inflammatory reaction process, histone modification often occurs. Histone acetylation in some regions is associated with disease activity. For example, acetylated histone H4, H2A and H2B become autoantibody targets in lupus [50]. On the other hand, histone acetylation in other regions seems to have protective effects [51]. Administration of histone deacetylase (HDAC) inhibitors led to reduced IL-6, IL-10, IL-12 and IFN-γ in splenocytes and improved glomerulonephritis in MRL-*lpr*/*lpr* mice [51]. Histone hyperacetylation has also been shown to be associated with the progression of SLE. Histone deacetylase sirtuin-1 (Sirt1) was over-expressed in MRL/lpr $CD4^+$ T lymphocytes. HDAC-treated cells contained autoantigens, which responded to SLE-derived autoantibodies [52]. Hyperacetylation of genes related to apoptosis or cell cycle regulation plays a role in SLE and LN [52]. In addition, genome-wide acetylation of histones seems to improve the symptoms in lupus-prone mice while hyperacetylation of various genetic loci in human SLE is associated with disease severity [53].

MicroRNAs

miRNAs are short non-coding RNA sequences that regulate gene expression by blocking protein translation or inducing mRNA degradation. The variation in miRNAs could cause the dysregulation of a broad range of targeting genes, which are associated with diseases. Therefore, miRNAs could be good biomarkers for autoimmune diseases [54]. The circulating miRNAs are systematically altered in SLE even LN [54]. The varied expression of miRNAs in kidney during pathological processes makes miRNAs a valuable new tool for understanding, diagnosing, and discovering therapeutic options for SLE and LN.

Dai et al. compared miRNA expression in renal biopsies of LN patients with normal controls by microarray technology and identified 30 down-regulated and 36 up-regulated miRNAs. These differentially expressed miRNAs may be potential diagnosis biomarkers to illuminate pathogenic mechanisms in LN [55]. Increased expression was detected for miR-142-3p and miR-181a in SLE patients, while expression of miR-106a, miR-17, miR-20a, miR-92a and miR-203 was decreased. Moreover, expression of miR-343-3p, miR-223 and miR-20a was significantly low in SLE patients with LN [54]. Compared to healthy controls, expression of miR-638, miR-198 and miR-146a was significantly different in LN patients [56]. Furthermore, the degree of change in glomerular miR-146a and tubulointerstitial miR-638 expression was correlated with clinical disease severity, suggesting that these miRNA targets may play a role in the pathogenesis of LN [56]. miR-371, miR-423, miR-1224, miR-663 and miR-638 were reported to be differentially expressed in LN across different racial groups. Among them, miR-371, miR-1224 and miR-423 were the first to be reported to be associated with LN [57]. Additionally, expression of miR-221 and miR-222 in urinary sediment was associated with LN disease activity and may also serve as biomarkers [58].

Profiling protein biomarkers in LN

The differential expression of protein biomarkers in serum and urine of SLE patients may reflect the pathophysiological status of disease development and therefore may be used as biomarkers for early diagnosis and prognosis. Common protein markers include antigens, autoantibodies and cytokines, *etc*.

Serum protein markers for LN

Autoimmune serology markers of SLE include anti-dsDNA antibodies, anti-C1q antibodies and complement C3 and C4 levels. Anti-dsDNA antibodies, first detected and measured 55 years ago, are the best studied of all autoantibodies found in SLE and are still ubiquitously used to help diagnose and manage this disease, because the level of this autoantibody is correlated with disease activity [59]. In addition, nucleosomes play a pivotal role in the development of kidney lesions by mediating the production and binding of autoantibodies to basal membranes [60]. Meta-analysis showed that anti-nucleosome antibodies are a highly accurate diagnostic marker for SLE and LN [61]. In addition, serum anti-C1q antibody is also a valuable noninvasive biological marker for prediction of renal histopathology in LN. The level of serum anti-C1q antibody can be used as a marker for LN activity with higher sensitivity and specificity than traditional markers of disease activity, such as C3/C4 consumption and anti-dsDNA [62].

Large-scale screening of the disease biomarkers using high throughput autoantigen microarray has facilitated the discovery of disease biomarkers at the global level. By using a glomerular proteome array, Li et al. identified autoantibody clusters that best predict the disease activities of SLE and LN [63]. Some of the autoantibody clusters, such as anti-chromatin, anti-DNA, anti-Ro and anti-RNP, were associated well with disease activity in SLE and incomplete lupus (ILE) [64]. They found that presence of IgM autoantibodies in patient's sera was associated with reduced LN severity [63,64]. These studies verified proteomic microarray as a powerful tool for uncovering novel autoantibody biomarkers for autoimmune diseases.

In recent years, some novel serum biomarkers for LN have been identified. For instance, serum a proliferation-inducing ligand (APRIL) as well as its intrarenal mRNA levels were reported to be associated with resistance to treatment, indicating that APRIL could be a potential biomarker for predicting difficult-to-treat cases of LN [65]. Panda et al. reported that higher and intermediate mannose binding lectin (MBL) levels are significantly associated with nephritis in SLE patients [66]. Since serum cystatin C is a good marker for renal function, the serum β2 microglobulin/cystatin C (Sβ2 M/SCysC) index could be a better indicator of renal activity in SLE [67]. In addition, serum complement factor H (CFH) levels are also associated with disease activity of LN [68]. Serum levels of soluble interleukin 7 receptor (sIL-7R) are strongly augmented in patients with LN, thus it may be a marker of SLE disease, especially nephritis [69]. A cross-sectional study including 20 patients suggested that vanin-1 level in peripheral blood may be a promissory biomarker for LN and warrants further validation in a larger cohort [70]. Other cytokines and chemokines have been proved to be associated with LN, including VEGF [71] and higher levels of IL-18 and IFNγ [72]. Increased levels of macrophage inflammatory protein-1α (CCL3), MCP-1, RANTES and IFNγ-induced protein-10 are also observed in the serum of SLE patients [73].

Urine Biomarkers for LN

Generally, urinary substances are likely to reflect kidney damage better than serum components. Urine is a source of biofluid which is easy to harvest and the biomarkers in urine are usually reflecting the renal function directly in various kinds of nephritic diseases. Proteomic approaches, such as two-dimensional gel electrophoresis, mass spectrometric and/or immunochemical identification of proteins, surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS) and capillary electrophoresis-MS, have been used to screen potential urine biomarkers that are associated with renal damages caused by LN [74]. The usual markers of renal disease include urinary protein, creatinine, *etc*., although the currently recommended spot urinary protein to creatinine ratio may not be sufficiently sensitive to detect early nephritis. Urinary biomarker candidates include adhesion molecules, cytokines, chemokines and their receptors [74]. The urinary proteome profiles of 129/Sv and DBA/1 mice that developed severe immune-mediated nephritis are significantly different from those of B6 and BALB/c mice [75]. For example, levels of vascular cell adhesion molecule 1 (VCAM-1), P-selectin, tumor necrosis factor receptor 1 (TNFR1) and CXCL16 were higher in the 129/Sv and DBA/1 strains, which were highly susceptible to severe immune-mediated glomerulonephritis. Another pilot study showed that transferrin, α1-acid-glycoprotein (AGP) and lipocalin-type prostaglandin D-synthetase (L-PDGS) may serve as potential biomarkers for impending nephritis flares in pediatric lupus [76].

More new urinary biomarkers have been reported. For example, urinary VCAM-1 level is associated with nephritis activity in SLE patients [77]. In addition, baseline mean urinary MCP-1 levels were significantly correlated with both LN class and severity of LN flare, hence, the high specificity makes urinary MCP-1 become a non-invasive marker for determining lupus flare and LN class [78]. Moreover, urinary free light chains (FLCs) are potentially useful biomarkers in International Society of Nephrology and Renal Pathology Society (ISN/RPS) class III/IV LN or proliferative LN [79]. Furthermore, urinary tumor necrosis factor-like weak inducer of apoptosis (TWEAK) levels were correlated with all active indexes of LN, suggesting its potential role as a novel biomarker of active LN [80]. Based on these studies, we summarized the protein markers in **Table 2**.

Conclusion

Over the last decade, omics-based techniques have been extensively utilized for biomarker screening at the level of genetics, epigenetics and proteomics. Reviewing the current list of best validated LN disease susceptibility candidate genes and the potential functional interaction of these genes may allow us to elucidate the genetic basis of LN predisposition. The effect of epigenetic regulation on LN pathology may also play an important role in the pathogenesis of LN. The variations at the genetic and epigenetic level as well as the environmental factors may work together to drive the pathogenesis of LN. The appearance and variation of protein biomarkers in serum

Table 2 Summary of protein markers associated with LN

Protein	Full name	Ref
Serum protein markers		
C3	Complement component 3	[81]
C4	Complement component 4	[81]
Anti-dsDNA Abs	Anti-double stranded DNA antibodies	[81]
Anti-C1q Abs	Anti-complement 1q antibodies	[62]
ESR	Erythrocyte sedimentation rate	[81]
CRP	C-reactive protein	[81]
APRIL	A proliferation-inducing ligand	[65]
MBL	Mannose binding lectin	[66]
Sβ2M/SCysC	Serum β2 microglobulin/cystatin C	[67]
CFH	Complement factor H	[68]
sIL-7R	Soluble interleukin 7 receptor	[69]
VEGF	Vascular endothelial growth factor	[71]
IL-18	Interleukin-18	[72]
IFNγ	Gamma interferon	[72]
CCL3	Macrophage inflammatory protein-1α	[73]
MCP-1	Monocyte chemoattractant protein-1	[73]
CCL5	Chemokine (C-C motif) ligand 5	[73]
IP-10	IFNγ-induced protein-10	[73]
VNN1	Vanin-1	[70]
Urinary protein markers		
VCAM-1	Vascular cell adhesion molecule 1	[75,77]
TNFR1	Tumor necrosis factor receptor 1	[75]
CXCL16	Chemokine (C-X-C motif) ligand 16	[75]
AGP	α1-acid-glycoprotein	[76]
L-PDGS	Lipocalin-type prostaglandin D-synthetase	[76]
MCP-1	Monocyte chemoattractant protein-1	[78]
FLCs	Free light chains	[79]
TWEAK	Tumor necrosis factor-like weak inducer of apoptosis	[80]

and urine of patients may reflect the pathophysiological status of disease development and therefore may be used as biomarkers for monitoring disease activity of LN.

As more emerging novel technologies, such as next-generation sequencing and high throughput proteomic arrays, are being widely employed in biomarker screening, we are expecting more and more biomarkers associated with lupus nephritis will be discovered. Elucidating the underlining molecular mechanisms between the biomarker dysregulation and disease phenotype will lead us to identify potential therapeutic targets for treatment of LN, and also to establish better tools for prediction, early diagnosis and prognosis of lupus nephritis.

Competing interests

The authors declare no competing interests.

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