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Review Article

The heterogeneity of systemic lupus erythematosus: Looking for a molecular answer

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ABSTRACT

The heterogeneity of SLE is a major limitation when designing clinical trials and understanding the mechanisms of the disease. The analyses conducted before the new technologies for the identification of the single cell transcriptome focused on the detection of molecular patterns such as interferon signature in total blood or through the analysis of major separate cell populations, such as CD4+ T cells. The analyses of molecular patterns have mainly focused on the transcriptome and DNA methylation changes. The first studies on single cell transcriptomics have now been published for mononuclear blood cells and tissues or the knowledge derived from them, total kidney, tubules and skin keratinocytes. The latter have defined patterns of nonresponse to treatment. However, much work still needs to be done to be able to use these methods in clinical practice.

La heterogeneidad del lupus eritematoso sistémico: buscando una respuesta molecular

Palabras clave:
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RESUMEN

La heterogeneidad del lupus es una limitante al momento de diseñar estudios clínicos, así como también para nuestra facultad de comprender los mecanismos de la enfermedad. Los análisis previos a las nuevas tecnologías para la detección del transcriptoma de célula única trabajaron en la identificación de patrones moleculares, como la firma del interferón en sangre total, o a través del análisis de poblaciones celulares principales separadas, como son las células T CD4^{+}. Los análisis de patrones moleculares se han enfocado primordialmente en el transcriptoma y en los cambios de metilación del ADN. Ya se han publicado los primeros

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estudios de transcriptoma de célula única para células sanguíneas mononucleares y para tejidos, riñón total, túbulos y queratinocitos de piel. Estos últimos han definido patrones de no-respuesta al tratamiento. Aún falta mucho para que los métodos o los conocimientos derivados de los mismos sean de utilidad en la práctica clínica.

Introduction

Systemic lupus erythematosus (SLE) is a highly heterogeneous disease, and this heterogeneity has had an enormous impact on how early the disease is diagnosed, how patients are treated or even on how the results of the research are reported. SLE is a disease with an insidious start that can present suddenly as a serious and life-threatening flare with several highly unspecific manifestations.

The use of molecular methods using blood cells or tissues for the stratification of the disease into meaningful molecular groups that represent the patient's mechanisms of disease, is still in its infancy. We depend on the clinical parameters at hand that rely on the organ-specific manifestations of disease; that is to say, when patients already exhibit varying degrees of organ damage. Classification criteria, disease activity indexes and damage indexes, which are often non-measurable, subjective and semi-quantitative parameters, hinder our ability to use molecular tools more accurately. The significant heterogeneity of SLE is mostly evidenced when it comes to clinical trials, when end points or response need to be defined.

This article is an update of the molecular tools available to classify lupus patients into clinically relevant groups. This is valuable information when using new biological drugs and will eventually show the molecular patterns of patients who will benefit the most from a given available drug, while also helping us in the discovery of new drugs. One of the most important advances has been the discovery and further confirmation of the type I interferon gene signature (IGS). The type I interferon signature is defined by the expression of a large group of genes that characterize downstream events of type I IFN signaling (IFN-I, mainly IFNa and IFNb), originally described in SLE[1,2] and later in other diseases.[3] In fact, while pediatric SLE patients show a high prevalence of both (70% of the patients have an interferon signature), adult patients do not, and in many instances, ethnicity and socio-economic aspects are also important in defining the type of disease they present.[4,5]

Therefore, predicting the type of molecular pathway that an SLE patient may develop becomes increasingly important. In fact, I would argue that clinical diagnoses limit the possibilities of defining an individual's molecular pattern, as many such patterns are related to inflammatory processes that could be shared across a large number of diseases and are not limited to autoimmune conditions. Actually, we may have to reconsider the term autoimmunity.

Along the same lines, we may even change the definition of normalcy, since an individual with a given molecular pattern of gene expression or epigenetic changes, may or may not be at risk of developing an inflammatory condition in the future. Hence, I would like to submit the term molecular diagnosis, a term that will need to be further refined as studies advance and technologies sharpen, particularly when studying patients and individuals prospectively. For the purpose of this review, I will limit my comments to SLE, including a discussion about the latest attempts to molecularly stratify the disease.

The blood molecular patterns in SLE

Transcriptome and epigenome analyses have been the major source of data which have been the basis for studies on disease stratification. Many studies have used blood, and others have used blood-derived cells, primarily T cells. When using blood, the major problem is signal dilution, if there is a cell-specific transcriptome difference that is searched for. So blood transcriptome analyses provide a general picture. Bradley et al. using transcriptome data from purified T cells identified subgroups of SLE patients according to disease severity.[6] However the number of patients was very small suggesting overfitting.

Flint[7] researched the interferon signature in further detail, primarily looking for differences in the type of signature expressed by various cell types (neutrophils, $CD4^+$ T cells, $CD8^+$ T cells and monocytes) across four different immune-mediated conditions, including SLE, and a healthy control group. These authors used the weighted gene expression network analysis (WGCNA), which identifies gene modules in the data based on co-expression (a method widely used after the publication of Chaussabel et al. in 2008[8]). For each cellular population a module was selected as the most representative for the interferon signature based on gene composition, its correlation with the diagnosis of SLE and the correlation with a 21-gene core interferon signature expression profile.

The authors[7] performed an extensive analysis of 1288 genes within the selected modules to compare between several autoimmune diseases and cellular populations. Four interferon modules comprising 67 genes were considered the core interferon genes, and most were found to be highly expressed in myeloid cells and neutrophils, while just a few, 11 genes, had increased expression in T cells. Interestingly, the higher expression of T-cell-specific modules appeared to be exclusive of SLE. On the other hand, monocytes and neutrophils presented similar expression levels in other diseases and in controls, supporting the idea that basal interferon signature levels are important to maintain myeloid populations, while this is not the case for T cells.

By separating various cell populations from SLE patients, McKinney[9] found that CD8+ T cells would show

two clinically relevant groups of SLE patients with different prognoses. A poor prognosis group is enriched for genes involved in the interleukin-7 receptor (IL-7R) pathway and T cell receptor (TCR) signaling and those expressed by memory T cells. Furthermore, the poor prognostic group is associated with an expanded CD8(+) T cell memory population.

DNA methylation is considered the most characteristic epigenetic mark.[10] Its presence or absence in different regulatory regions of the genome has been associated with changes in gene transcription, chromosome stability, or the regulation of alternative splicing.[11] Aberrant patterns of DNA methylation have been involved in the pathogenesis of autoimmune diseases and changes may be related to genetic loci through meQTLs.[12] The specific expression of T cell modules in patients with SLE is consistent with hypomethylation findings of type I interferon-regulated genes in naive $CD4^+$ T cells[13] suggesting that prior to stimulation these cells are poised to express type I IFN inducible genes.[14]

Routine clinical practice could benefit from a comparison among different autoimmune diseases to identify discriminant profiles. With this goal in mind, one study analyzed SLE and rheumatoid arthritis (RA), and classified both diseases into 3 groups with specific profiles that overlapped between both diseases.[15] Interestingly, they replicated the association between RA and SLE in later studies,[16] but were not able to replicate the groups, most probably because of the small sample size. The study showed that early RA patients grouped better with SLE than RA patients with longer disease duration.[17] The shared gene signature affected B-cell function,[18] suggesting that these individuals could eventually be treated with B-cell-targeted therapy; however, the study also suggests that the earlier the disease the stronger the similarities and possibly the easier to treat with therapies such as Rituximab.

Another study that analyzed SLE and systemic sclerosis (SSc) transcriptomes together found that 62% of differentially expressed genes in SSc versus healthy individuals were also differentially expressed when analyzing SLE.[19] Type I IFN inducible and JAK/STAT signaling pathways were enriched, as well as pathogen pattern molecular recognition functions. Some SSc patients grouped with SLE. These "lupus-like" patients had increased type I IFN inducible and plasma cell gene expression, and were therefore highly similar to SLE. In both diseases, the increased expression of IFN-inducible genes was related to disease activity and with the presence of antinuclear antibodies.[19,20] The type I IFN signature was also associated with subsets of RA.[21] A study searching for shared signatures between autoimmune diseases[22,23] showed that genes differentially expressed between diseases and controls were also common across diseases, primarily, but not exclusively, the interferon-signaling pathway. From the clinical point of view, this information could be important for the potential use of anti-IFN receptor therapy (i.e. Anifrolumab) even for subsets of RA and SSc once the molecular patterns characterizing the different patients are available.[24]

General DNA hypomethylation in various cells and tissues has been observed with similar patterns in various diseases. This was observed in RA and SLE in T-lymphocytes, synovial tissue, synovial mononuclear cells and peripheral blood.[25,26] In genome-wide studies, this global T-lymphocyte hypomethylation was confirmed for SSc and Sjogren's syndrome (SjS),[14,27] but not in dermatomyositis.[27] In SLE, SSc, and SjS the hypomethylated pattern was observed in B- lymphocytes, monocytes, dermal fibroblasts and leukocytes.[28–30] These patterns correlated with a decreased expression of DNA methylation machinery genes *DNMT1*, *DNMT3B* or *MBD4*. In SLE and SjS hypomethylated genes are enriched with the type I interferon pathway genes.[14,28,31] It is interesting to mention that methylation machinery genes are dependent on environmental factors such as dietary folates.[32,33] On the other hand, the variety of cells showing the type I IFN signature suggests that whichever inducer there is, it is systemic, possibly viral. A recent study showed that 50% of the promoters of known genetic risk loci of SLE are occupied by the Epstein-Barr virus EBNA2 protein, many of which co-cluster with other human transcription factors. This is a first example of a gene-environment interaction with direct potential epigenetic regulation and conditioning the effects of genetic risk loci by a viral transcription factor.[34]

The *IFI44L* promoter is hypomethylated in SLE patients and is the most hypomethylated site among interferon-inducible genes. When comparing with RA and SjS, despite the *IFI44L* promoter being hypomethylated in all diseases, the methylation levels distinguished SLE from the other.[35] We recently observed that *IFI44L* exhibited highest methylation variability in patients with mixed connective tissue disease (MCTD) and could not differentiate MCTD from SLE[36] suggesting that this gene may not be useful to distinguish between both diseases. Another study compared DNA methylation in monozygotic twins discordant for three diseases (including SLE and RA). The authors only found 49 genes with significant differences in DNA methylation.[30]

One issue that is of major interest is what is the gene expression response during disease activity and if this may stratify patients or reveal different patient groups or classes that may help us sort out the heterogeneity of SLE.

With this in mind, Banchereau et al.[37] used for the first time longitudinal total blood gene expression data identifying 7 groups of pediatric SLE patients where specific gene expression modules were associated with each cluster.[37] They also identified the relationship between neutrophil gene expression and lupus nephritis.

Once again WGCNA was used.[8] The authors selected the most correlated the SLEDAI of each patient at each time point projecting their expression profiles to the Chaussabel modules.[8] The 7 groups of patients described corresponded to 5 immune signatures that were different from each other in terms of the types of cellular mechanisms: lymphoid, erythropoiesis, plasma cell, neutrophil/myeloid, and type I IFN. However at least 3 of the groups of patients had an IFN module.

One group of patients where all had nephritis correlated with the IFN, neutrophil and plasmablast-associated modules. These patients had anti-dsDNA antibodies and the most severe clinical disease. The neutrophil and IFN signatures correlated strongly with development of lupus nephritis and were modified by Mycophenolate Mofetil (MMF) treatment, particularly in patients with proliferative rather than membranous glomerulonephritis.

Using the data from Banchereau et al., and an extra set of adult SLE patients from Johns Hopkins[38] we took a

somewhat different approach. Individual genes were selected by their correlation with the SLEDAI, followed by clustering. This study gave 3 clusters that were replicated in the adult set. The WGCNA was then used to investigate the functionality of the genes within each cluster. Importantly, the SLEDAI is a semi-quantitative score with many drawbacks and that a continuous score based on real measurements that may be correlated with transcriptome data in a longitudinal fashion would be the ideal. The three clusters had particular characteristics: cluster 1 was heterogeneous and contained features of clusters 2 and 3, but the patients from that cluster could not be assigned to any of the other two. Cluster 2 was clearly differentiated and showed a sharp relationship with the type I IFN signature. This means that during disease activity, during increases in the SLEDAI scores, the type I IFN signature genes correlated positively with the score. Not all genes of the IFN signature showed this correlation. There was also a correlation with increases in levels of neutrophils, C3 and the ESR, and a negative correlation with levels of lymphocytes. In adult data, this cluster was also associated with lymphopenia. On the other hand, cluster 3 was completely opposite, where lymphocytes showed a positive correlation with the SLEDAI and a negative correlation between the SLEDAI and neutrophil numbers or proportions.

Interesting differences were observed from the clinical point of view: patients from cluster 1 had the highest risk to develop proliferative nephritis compared with cluster 3. This was particularly obvious in adult patients where 65% of patients from cluster 1 were at risk to develop proliferative nephritis against 13% of those from cluster 3. Patients from cluster 3 showed features of skin disease, antiphospholipid syndrome and increased liver enzymes. Patients from cluster 2 also showed an increase in development of lupus nephritis; however, it will be interesting to determine whether there is a difference in terms of the underlying pathology in nephritis.

Disease activity did not condition the clusters, and indeed, there were no differences in the SLEDAI components or in the magnitude of the components between clusters. Moreover, treatment validation showed that therapy did not influence the allocation of patients to the clusters, and again, no differences in treatment between the groups were observed. Furthermore, since neutrophils seem to be important in defining the clusters, there were no differences in neutrophil numbers between the groups when analyzed following treatment. In fact, when performing a feature (gene) selection against treatment doses, for each type of therapy for which the data was available, only 2% of the genes selected by treatment overlapped with the genes selected when correlating with the SLEDAI and forming the clusters. In summary, the clusters had molecular patterns that most likely reflected the drivers of disease activity, being primarily differentiated by cell types: neutrophils on the one hand, and lymphocytes on the other. This study suggests then a simple approach, the use of a neutrophil:lymphocyte ratio (NLR). Indeed, a study has shown that increased neutrophils and hence increased NLR is associated with more disease activity and associated with dsDNA antibodies and the presence of the IFN signature. Interestingly, the authors also found that high neutrophil count was associated with neutrophil activation markers, such as calprotectin, whereas low lymphocyte count was associated with type I IFN activity and elevated numbers of low-density granulocytes (LDGs).[39]

It is certainly possible that once the groups have been stratified into molecular blood patterns, patterns in tissues may create a sub-stratification of each cluster, and such sub-stratification may be important in the treatment of, for example, lupus nephritis. I believe this would be the case in SLE, particularly with the differences we observe in the risk to develop nephritis in clusters 1 and 2 or even cluster 3, a cluster formed by individuals with secondary Sjögren's syndrome and anti-phospholipid syndrome and who may develop other pathological classes of glomerulonephritis.

One important point here is that patients having an IFN signature in a one-time window do not necessarily respond with changes in expression of interferon genes during moments of disease activity or inactivity. Thus, simply identifying patients with high or low IFN gene signature expression at one time point may not be reflecting the reality of the process occurring during flares.

A second study[40] compared the SLE gene expression data with drug-induced gene signatures from the CLUE database in order to compute a connectivity score that would reflect the capacity of a drug to revert patient signatures. This comparison revealed robust clusters identical to those previously obtained with the patients' gene expression data suggesting that the use of differential treatment may depend on the cluster to which a patient belongs. The best drug candidates found were mTOR inhibitors or those reducing oxidative stress as these showed the stronger cluster specificity, as previously shown. These drug patterns follow the cell-specificity of the clusters, and interestingly, although not significant, suggested to a certain extent the possible causes of non-response, that is, using the wrong drug on the patient with a cluster not compatible with that drug.

Analyzing jointly transcriptome and methylation patterns from blood, Barturen et al.[41] were able to group 7 different diseases, namely SLE, RA, Sjögren's syndrome, systemic sclerosis, primary antiphospholipid syndrome and mixed connective tissue disease, as well as patients with undifferentiated connective tissue disease (UCTD), and grouped the diseases into 4 clusters. Three of the clusters were found during active disease and were considered pathogenic and represented an inflammatory cluster, a lymphoid cluster and an interferon cluster. The fourth cluster was undefined, incorporated cases with low disease activity and several systemic sclerosis and RA patients. Interestingly, when looking into an inception cohort that was followed prospectively for 6 and 14 months after recruitment, the allocation to the clusters was stable and if an individual changed cluster it was almost exclusively to the undifferentiated cluster, and then back to their pathological cluster, supporting the identity of this cluster in individuals in remission. For example, a patient belonging at first to the lymphoid cluster, could at 6 months belong to the undefined cluster and at month 14 was again in the lymphoid cluster. This was observed in around 34% of the cases followed throughout the process. The interferon cluster was associated with some of the most extreme phenotypes such as kidney function abnormalities (including nephritis), thrombosis, nervous system involvement, and leukopenia, in addition to other minor comorbidities. Fibrosis complications in both skin and

the musculoskeletal system were accentuated in the inflammatory cluster, in addition to kidney related clinical features. The lymphoid cluster, in general, presented less aggressive phenotypes than the other clusters, with increased dyslipidemias, presence of gastrointestinal manifestations, such as abdominal pain, diarrhea, and constipation. Association with *sicca* syndrome was also found in this cluster. The interferon cluster showed an enrichment of anti-dsDNA, anti-Sm, anti-SSA, anti-SSB, anti-U1RNP and protein free light chains, and an increase in IP-10, BAFF, MCP-2 and TNF-α; the inflammatory cluster was increased in MMP-8 and C-reactive protein; while high levels of IL-1RA and CXCL13 were shared by the inflammatory and interferon clusters. As expected, the interferon cluster was dominated by SLE and Sjögren's, but also MCTD and some UCTD, RA and systemic sclerosis, while the inflammatory and lymphoid clusters had all diseases represented more or less in the same proportions. In summary, the clusters have specific clinical and serological characteristics, but also quite different regulatory and genetic landscapes that are beyond the scope of the discussion, but may suggest 3 alternative and different molecular pathways that could define each individual patient's illness.

The blood-derived single cell molecular transcriptome patterns

Technology has now come to a point where we can assess the transcriptome of a single cell. Only one study has been published for peripheral blood. These studies allow to define exactly in which cells the abnormalities reside and possibly the unique cell mechanisms behind the abnormality. The paper by Nehar-Belaid and Hong (contributed equally)[42] describes the analysis of peripheral blood mononuclear cells but not of neutrophils. The main reason for this is that neutrophils' RNA degrades rapidly and is difficult to obtain for appropriate transcriptome analysis in the method used, 10x-Genomics. As before, the type I interferon signature is widely expressed, but interestingly, the interferon signature was observed in defined subpopulations of the major cell types, including plasma cells. These analyses require clustering of the cell populations into genes expressed to achieve differentiation and occasionally to identify potentially new populations of cells. The groups or clusters are then named somewhat arbitrarily. The cells primarily expressing the interferon signature genes were: the major monocyte cluster (named C0) and all seven minor clusters, C11 (a minor CD4+ T cell cluster); C13 (CD16+ monocytes), C15 (megakaryocytes), C16 (cDCs), C17 (PCs), C18 (pDCs), and C19 (ISGhiGzK+acCD8+ T cells). Some genes allowed to group the patients into 9 groups called G1-G9: G1 was the group where type I interferon genes were most upregulated and were featured by *ISG15* and *IFI27*; G2 by *IFI35* and *ADAR*; G3 by *IFI44L* and *PAPR9*; G4 by *TMEM140* mainly upregulated in megakaryocytes; the G5 genes were *IFNGR1*, *CASP1* and *FCGRIA*, which were primarily expressed in monocytes, DCs and megakaryocytes; G6 genes were *CCL4*, *CCL5* and *IFNG* and were upregulated in NK cells, C3_GzH_acCD8+ T and C19_ISGhi_GzK+_acCD8+ T cells; G7 had primarily *SOCS1* upregulated and found in pDCs but also in subsets or clusters of T cells; G8 included *IRF7* and *IFNLR1* found in plasma cells in SLE patients, and G9 genes had the *IFNAR* genes found in PCs.

Many of the populations showed similar upregulation in patients and controls, revealing how these genes are normally expressed in specific cell types, but others were clearly upregulated in SLE and cell subsets expanded. The authors analyzed genes that co-expressed in the cell populations with the major genes defining the clusters; they found that in monocytes with high interferon signature, genes also coexpressed *IL1B*. Cells co-expressing *IL1B* still could be divided into three signatures of cells: one cell group co-expressing *IFITM3* and *IL1B*, another *ISG15* and *IL1B* and a third one, had both of them (10% of the cells).

Each of the major cell populations were finely characterized as well as signatures most associated with disease activity. For instance, *ISG15* was overexpressed in CD14+ monocytes in patients with high disease activity. Interestingly, *ISG15* expression was restricted to a B cell subpopulation that the authors named as B-SC5, which was the only cell type expressing *TBX21* (the transcription factor T-bet) and other genes expressed exclusively in this cell type suggesting that these corresponded to the ones previously described as extrafollicular, autoreactive, also called DN2 B cells.[43] Those genes are *ITGAX* (CD11c), *FGR*, *TFEC*, *FCRL2*, *FCRL3* and *FCRL5*, and *IL10RA*.

One type of pDC, named PDC-SC1 was expanded in SLE and contributed to the interferon signature. On the other hand, only one DC population was expanded in SLE and did not show any disease activity difference. The expanded cell cluster was named DC-SC2, which corresponded to the AXL+SIGLEC6+ DCs, the main subset expressing interferon signature genes. Finally, the T cell subsets mainly expressing interferon signature genes were the cytotoxic T cells, namely the T-SC4 cells. These cells were particularly expanded in SLE and included CD4+T cells that mapped to the ISGhiC11_CD4+T cluster, in turn expanded in SLE.

A specific cluster of NK cells, which was ISGhi was also specific for SLE. Two NK clusters, NK-SC2 and NK-SC3 were expanded in SLE and both had upregulated interferon and cytotoxicity-encoding genes with a particular increase of ISG15 and CD56 in the NK-SC2 subset.

Because the cohort used for the single cell study comprised children with SLE, primarily, the authors also analyzed the expression of genes mutated in SLE-related monogenic diseases, such as complement components *C1Q*, *C1R* or *C2*, *TREX1* or *DNASE1L3*, among others. The authors found that most of these genes although with the exception of the complement genes, were expressed primarily in pDCs and DCs, but also in plasma cells, the B- SC5 subset, and the NK-SC3 subset.

Finally, the authors used the single cell abundance to classify SLE patients into groups, validating the groups in a second adult cohort, revealing similar expansions of cellular populations that had associations with disease severity.

Single cell transcriptome in tissues

The AMP RA/SLE Consortium published two papers that performed single cell transcriptome sequencing of two main tissues: the kidney and the skin of lupus patients. The study analyzing skin did so together with the kidney data, showing

a certain reflection of kidney differentially expressed genes in specific cell types in the skin.

Arazi et al.[44] performed a single cell analysis of lupus kidneys and identified 21 subsets of infiltrating cells with pro-inflammatory responses. The authors also found a high correlation between the renal and the systemic interferon response. Interestingly enough, they also identified local B cell activation with of a subset of B cells showing a signature similar to that of pro-inflammatory cells also called age associated B cells, somewhat similar to the DN2 mentioned above. Most of the cells showed an interferon gene signature and in particular two chemokine genes were broadly expressed, CXCR4 and CX3CR1, suggesting an important role of cell trafficking. The authors also analyzed urine cells and found an important correlation between these and the gene signatures found in the kidney. This has important clinical implications in that urinary cell markers could be used instead of kidney biopsies.

Der and cols,[45] analyzed kidney and skin biopsies in conjunction. The authors focused more on the kidney tubular cells and identified an interferon signature in these cells and in keratinocytes of the skin. They also saw a fibrotic signature in the tubular cells associated with failure to respond to therapy, in a way similar to what was found in synovial tissue in rheumatoid arthritis.[46] This is very interesting because it suggests that some of the nephritis might not be immunologic or a rapid fibrotic process. In the case of the lupus tubular cells, the genes *TIMP1* and *SERPING* were shown, in a pathway analysis to be extracellular matrix-interacting molecules. The authors also noted in the non-responders an upregulation of the complement and coagulation cascades, including the genes *C1S* and *C1R*. A similar pattern was observed in keratinocytes in non-responders. In this case, the non-responders also showed some collagen genes that partly overlapped with those found in the kidney, such as *COL1A1* in both tissues, or *COL17A1* only in keratinocytes. Using a predictive model authors found that the expression of four genes, *COL1A1*, *COL14A1*, *COL1A2*, and *COL5A2* could predict response to treatment with an accuracy of 92% (AUC of .96). However, the signature fibrotic scores found did not correlate with other disease activity or chronicity scores. The authors also could not correlate the fibrotic gene expression profiles with the histological pattern of fibrosis using light microscopy. In addition, PBMCs of the same individuals did not show any fibrotic markers. Looking into infiltrating cell receptor-ligand interactions, interactions between infiltrating leukocytes and potential ligands within tubular cells was found. For example, genes such as the fibrosis receptor *FGFR3* were expressed in tubular kidney cells, while its ligand *FGF13* was found expressed in leukocytes. Authors then analyzed separately biopsies of proliferative, membranous and mixed nephritis and identified different inflammatory pathways. Based on the observations, in proliferative nephritis keratinocytes upregulated TNF and the interferon pathways, but this was not the case in membranous nephritis. In the tubular cells, the proliferative class had an upregulation of *TNFR1* signaling.

Conclusion

The very first studies using single cell transcriptome analysis in peripheral blood, kidney and skin in SLE have been published, and the studies provide granularity to the data previously described. It will be exiting to further understand how the single-cell studies help in dissecting the heterogeneity of SLE in the follow up of patients during periods of relapse and remission and treatment response. The combination of both, tissue and PBMC, in a longitudinal approach will be essential to better understand how to use these methods to classify patients with SLE, and how blood may reflect or not tissue patterns of gene expression following therapy and possibly during clinical trials. The methods to discern cell types are still being developed, but the development occurs quite rapidly and swiftly. Projects such as the AMP RA/SLE project of the NIH (https://www.nih.gov/research-training/accelerating-medicines-partnership-amp), or the European 3TR (www.3tr-imi.eu) will be fundamental for a comprehensive understanding. The AMP Consortium has been publishing seminal papers that represent important advances in our understanding of such a complex disease as SLE.

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Conflict of interest

None.

Appendix A. Supplementary material

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