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Splicing factor SRSF1 acts as a novel molecular brake for CD4 T cell activation and controls key molecular pathways in SLE

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Abstract

T cells from systemic lupus erythematosus (SLE) patients exhibit a hyperactive phenotype with defects in homeostasis, signaling and cytokine production. We previously uncovered new roles for serine arginine-rich splicing factor 1 (SRSF1) in the control of genes involved in signaling and cytokine production in T cells. SRSF1 expression is decreased in T cells from patients with SLE and low SRSF1 levels are associated with severe disease activity. Mice with a T cell-conditional deficiency of Srsf1 exhibit T cell hyperactivity, systemic autoimmunity, and lupus-like nephritis. However, little is known about the molecular targets controlled by SRSF1 and whether they are implicated in human SLE. Our goal was to identify the molecular signatures controlled by SRSF1 and evaluation by comparative bioinformatic analysis if these genes and pathways are dysregulated in SLE. We curated publicly available gene array datasets from SLE patients and compared them with SRSF1-regulated genes in CD4 T cells from Srsf1-deficient mice. We identified 169 overlapping genes controlled by SRSF1 that are aberrantly expressed in T cells of SLE patients. Pathway analysis revealed genes enriched in interferon signaling, cytokine production, cytokine receptor interaction, cell migration and lysosomal clearance pathways. Our data reveal that SRSF1 controls genes involved in T cell homeostasis, activation, cytokine regulation/signaling and differentiation, which are altered in patients with SLE. Therefore, SRSF1 is an important regulator of T cell function and its deficiency may lead to a hyperactive T cell phenotype in SLE patients. Targeting SRSF1 and the genes controlled by this molecule to correct the aberrant T

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Significance statement: In this study, we identified 169 overlapping genes controlled by SRSF1, which were aberrantly expressed in patients with SLE. Pathway analysis unveiled that these genes were enriched in interferon signaling, cytokine production, cytokine receptor interaction, cell migration, and lysosomal clearance pathways. The findings also showed that SRSF1 plays a crucial role in regulating genes involved in T cell homeostasis, activation, cytokine regulation/signaling, and differentiation, all of which were found

to be altered in patients with SLE. Moreover, the study suggested that SRSF1 plays an important role in regulating T cell function and its deficiency may lead to a hyperactive T cell phenotype in patients with SLE. These findings shed light on the potential mechanisms underlying the development of SLE and highlight potential of SRSF1 as a therapeutic target for treating SLE.

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ABSTRACT

T cells from systemic lupus erythematosus (SLE) patients exhibit a hyperactive phenotype with defects in homeostasis, signaling and cytokine production. We previously uncovered new roles for serine arginine-rich splicing factor 1 (SRSF1) in the control of genes involved in signaling and cytokine production in T cells. SRSF1 expression is decreased in T cells from patients with SLE and low SRSF1 levels are associated with severe disease activity. Mice with a T cell-conditional deficiency of *Srsf1* exhibit T cell hyperactivity, systemic autoimmunity, and lupus-like nephritis. However, little is known about the molecular targets controlled by SRSF1 and whether they are implicated in human SLE. Our goal was to identify the molecular signatures controlled by SRSF1 and evaluation by comparative bioinformatic analysis if these genes and pathways are dysregulated in SLE. We curated publicly available gene array datasets from SLE patients and compared them with SRSF1-regulated genes in CD4 T cells from Srsf1-deficient mice. We identified 169 overlapping genes controlled by SRSF1 that are aberrantly expressed in T cells of SLE patients. Pathway analysis revealed genes enriched in interferon signaling, cytokine production, cytokine receptor interaction, cell migration and lysosomal clearance pathways. Our data reveal that SRSF1 controls genes involved in T cell homeostasis, activation, cytokine regulation/signaling and differentiation, which are altered in patients with SLE. Therefore, SRSF1 is an important regulator of T cell function and its deficiency may lead to a hyperactive T cell phenotype in SLE patients. Targeting SRSF1 and the genes controlled by this molecule to correct the aberrant T cell phenotype may lead to potential novel therapeutics.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a debilitating chronic systemic autoimmune disease, which afflicts women in the childbearing years and is among the leading causes of mortality in young women (Dall'Era, 2013). Aberrant gene regulation, signaling and function of T lymphocytes are key features of immune dysregulation in lupus patients and mice (Katsuyama et al., 2018; Moulton and Tsokos, 2015), and are therefore potential therapeutic targets and biomarkers for disease management. Delineating the molecular signatures and pathways underlying defective immune cells and the molecules which control them is critical to identifying new molecular targets. RNA sequencing/transcriptomic profiling approaches have enabled the identification of molecular signatures underlying the defects in immune cells in various autoimmune diseases including SLE.

T cells from patients with SLE exhibit several signaling and gene regulation defects, which contribute to their hyperactivity and dysfunction (Katsuyama et al., 2018; Moulton and Tsokos, 2015). For example, individuals with SLE have reduced transcript of CD3 zeta chain resulting in defective proximal T cell receptor (TCR) signaling and their hyperactivity (Kammer et al., 2002). Recruitment of alternative downstream signaling molecules such as Syk kinase lead to increased strength of signaling with increased calcium flux (Clements and Koretzky, 1999; Kammer et al., 2002). Cytokine defects include low IL-2 production and increased inflammatory cytokines such as IL-17 have also been noted in T cells and have established roles in SLE pathogenesis (Alcocer-Varela and Alarcon-Segovia, 1982; Bengtsson et al., 2000; Laurence et al., 2007; Linker-Israeli et al., 1983; Lourenco and La Cava, 2009; Ytterberg and Schnitzer, 1982). Additionally, an elevated type I interferon signature has been shown in SLE patients (Alcocer-Varela and Alarcon-Segovia, 1982; Bengtsson et al., 2000; Laurence et al., 2007; Linker-Israeli et al., 1983; Lourenco and La Cava, 2009; Ytterberg and Schnitzer, 1982) which impacts multiple cells of both the innate and adaptive immune systems. Yet the molecules that control these global programs of TCR activation, differentiation, cytokine production and cytokine signaling are not fully known.

Serine arginine rich splicing factor 1 (SRSF1) is the prototype member of the SR family of splicing factors and is a key controller of constitutive and alternative splicing events (Das et al., 2013). While SRSF1 has

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been shown to control genes involved in cell survival (Maslon et al., 2014), its roles in T cells or the immune system and in autoimmune disease are virtually unknown. By discovery approaches of oligo-pulldown and mass spectrometric proteomics screening, we identified SRSF1 binding the mRNA of the TCR-associated CD3 zeta chain and showed that SRSF1 positively regulates its expression in human T cells (Moulton et al., 2015; Moulton et al., 2014; Moulton et al., 2008; Moulton and Tsokos, 2010). We demonstrated that SRSF1 controls genes involved in T cell signaling and cytokine production in human T cells (Moulton et al., 2015; Moulton et al., 2013; Moulton and Tsokos, 2010). Furthermore, we found that T cells from SLE patients exhibit low levels of SRSF1, which is associated with severe disease (Moulton et al., 2013); and importantly, overexpression of SRSF1 rescues IL-2 production in SLE T cells (Moulton et al., 2013). Furthermore, we have recently shown in proof-of-concept in vivo studies that selective deletion of SRSF1 in T cells in mice leads to T cell hyperactivity and lupus-like systemic autoimmune disease (Katsuyama et al., 2019; Katsuyama et al., 2021; Katsuyama and Moulton, 2021). We have also shown that SRSF1 is necessary for T cell homeostasis and its deficiency correlates with comorbidities including lymphopenia in SLE patients (Katsuyama et al., 2020). While we have identified by transcriptomics profiling a large number of putative genes and pathways controlled by SRSF1 in T cells from mice (Katsuyama et al., 2019), the relevance of these molecular signatures in the context of SLE pathogenesis remains unknown.

The etiology of SLE is thought to be from an interplay between genetics, environmental and hormonal factors. Besides rare cases of monogenic lupus as seen with specific complement deficiencies, multiple genes may influence an individual's risk of developing SLE along with environmental triggers. High-throughput sequencing and transcriptomic profiling can be used to quantify the expression levels of large numbers of genes simultaneously. When combined with bioinformatic analysis and supplementation with data from specific genetically engineered animal models, one can delineate key pathways controlled by these specific pathogenic genes in complex diseases.

Our recent studies have shown that T cell conditional SRSF1-ko mice develop lupus like disease due to aberrant T cell function. We have uncovered differentially expressed genes in these mice. In this study we sought to examine the various genes that are differentially expressed in CD4 T cells in individuals with SLE and identify pathways that are possibly regulated by SRSF1. To this end, we performed comparative bioinformatic analyses between transcriptomic profiles of CD4 effector T cells from Srsf1-knockout (KO) mice with transcriptomes of CD4 T cells from patients with SLE. We further performed pathway analysis along with RNA – protein interaction predictions to determine genes controlled by SRSF1 in patients with active SLE.

METHODS

Transcriptomics profiling dataset from Srsf1-ko mice

We used previously generated transcriptomics profiling data from effector CD4 T (Teff) cells from Srsf1-ko mice (Moulton and Tsokos, 2015). Teff cells were generated by stimulating naïve CD4 T cells from spleens of *Srsf1* -T cell-ko or control WT (n=3) mice for 72h with CD3/CD28 antibodies. RNA from CD4 T effector cells was subjected to RNA-sequencing (R-seq). DAVID Bioinformatics Resource 6.8 Gene ID Conversion was used to convert mouse to human gene names.

Transcriptomics datasets from SLE Patients

To identify publicly available gene array data in CD4+ T lymphocytes from patients with SLE, we used the NCBI gene expression omnibus (GEO) 2R datasets, PubMed, and Google Scholar databases. Studies that did not use microarray or RNA sequencing were excluded from our search. Studies that used naïve CD4+ T cells or only whole blood and peripheral blood mononuclear cells (PBMC) without isolation of CD4+ T cells were also excluded. We used the search word "Lupus" in (GEO)2R and identified two datasets (GSE51997 and GSE4588) that matched our criteria. We included 6 active SLE (SLEDAI range 6-22, ANA positive) and 4 healthy controls from GSE51997 and 8 active SLE and 10 healthy controls from GSE4588. Next, we used the search words "Lupus RNA seq" in PubMed and identified one dataset (Crow, 2014) that matched our criteria for active SLE (n=15) and 10 healthy controls ($p<0.05$). Finally, we used Google Scholar and

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searched the keywords "RNA seq", "T cells", and "Lupus" using timeline limits between 2008 till 2019. Two studies fit our requirements and we included them in our study (Liu et al., 2020). From one study (Reyes et al., 2019), we used 5 active SLE and 5 healthy controls ($p<0.05$). The Liu study had analyzed individuals with SLE according to organ involvement. We included from this study, 4 SLE (skin), 4 SLE (renal and skin symptoms), 4 SLE (renal and joint symptoms), and 4 healthy controls (p[?]0.001). In total, 46 active SLE and 33 healthy controls from the five datasets from our search were included in our analysis.

Comparative bioinformatics data analysis of transcriptomics data

To compare the DEGs derived from RNA-sequencing data from the T-cell-*Srsf1* -cKO mice and from gene array datasets from SLE patients, we used the MIT comparison tool BaRC (*http://barc.wi.mit.edu/tools/compare/*). Next, comparative analysis of mouse and human gene array data was performed using Metascape (*http://metascape.org/gp/index.html#/main/step1*) to identify overlapping gene signatures in SLE patients controlled by SRSF1. Data was analyzed for differentially expressed (DE) genes, gene set enrichment, Kyoto encyclopedia of genes and genomes (KEGG) and gene ontology (GO) pathways. In addition, omicX FunCoup tool (*https://omictools.com/funcoup-tool*) was used for analysis of protein-protein interactions (Figure 3c). STRING tool (*https://string-db.org/*) was used to identify co-expressed genes for Srsf-1 and our mouse and human gene lists (Figure 3d). Both STRING and FunCoup were used to visualize interactomes between mouse and human gene array data (Figure 3c). (GEO)2R Profile Graph was used for further analysis of Srsf1 gene expression levels in GEO dataset GSE51997. RPINbase (*http://rpinbase.com/Explore*) was used for RNA -protein interactions.

RESULTS

Workflow Set-up and datasets curated for comparative bioinformatics analysis

We have recently described new roles for the splicing factor SRSF1 in the control of genes involved in signaling and cytokine production in T cells and SLE (Moulton et al., 2015; Moulton et al., 2014; Moulton et al., 2013; Moulton and Tsokos, 2010). We have also generated novel T cell conditional Srsf1-KO mice which demonstrates SRSF1 as a novel regulator of immune-response-related genes and pathways in CD4 effector T cells from T cell conditional Srsf1-KO mice (Moulton and Tsokos, 2015). These mice develop T cell hyperactivity, systemic autoimmunity and lupus like disease (Moulton and Tsokos, 2015). In this study we evaluated by comparative bioinformatics analysis of transcriptomic data, pathogenic pathways that are likely controlled by SRSF1 in SLE. Figure 1 shows a schematic of the workflow used for data analysis. Table 1 displays the five SLE patient datasets selected for analysis. RNA-sequencing was done in effector CD4 T cells from mice to evaluate genes controlled by SRSF1 (Katsuyama et al., 2019). The mouse effector CD4 T cells RNA-sequencing data analysis yielded 612 DE genes (Figure 2A) compared to control mice at the 2-fold cutoff with a p value<0.05. Of these, 312 genes were significantly upregulated and 300 genes were downregulated.

Identification of differentially expressed genes (DEG) in CD4 T cells in SLE patients

We analyzed publicly available gene array data from CD4 T cells in a total of 46 individuals with SLE and 33 healthy controls. The NCBI gene expression omnibus (GEO)2R database and published research in the literature were used to curate publicly available gene array datasets in SLE patients. Of these, only studies utilizing CD4 T cells were included for analysis. Ultimately, a total of five datasets, three using microarray technology and two utilizing RNA-sequencing were included for analysis. Following data normalization, preprocessing, and filtering with the criteria of adjusted $p<0.05$ and $|\log_2 FC|>0.8$, a total of 4179 genes were identified as differentially expressed in CD4 T cells of patients with SLE compared with healthy controls. Among these, 2306 were upregulated and 2002 were downregulated (Figure 2b). The overlapping differentially regulated genes in SLE patients and SRSF1 KO mice were evaluated to identify those that may be regulated by SRSF1 in humans (Figure 2c) and the relationship between adjusted p value (<0.05) and log2 fold expression of overlapping genes was plotted (Figure 2d).

Identification of common genes and pathways in CD4 T cells controlled by SRSF1 and impli-

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cated in SLE patients.

We first converted DEGs identified in Srsf1-KO mice to their human homologues (Supplementary table 1). 41 genes from the upregulated group and 30 genes from the downregulated group were excluded since these genes were exclusive to mice and not found in humans (Supplementary table 2). Next, we filtered the DEGs from human SLE patients and the human homologues from SRSF1 KO mice for adjusted p value of <0.05 and separated the genes that were shared in these two data sets. From this analysis, we were able to identify 73 genes that were upregulated in CD4 T cells of Srsf1-KO mice and in individuals with SLE while 42 genes were downregulated in both datasets. These genes are listed in Supplementary table 3. To identify the pathways represented by these DEGs in mice and humans, we performed gene ontology (GO) analysis (Figure 3). The most significant biological processes (BP) represented by these DEGs involved the immune system particularly those pertaining to immune responses and metabolism. Specifically, differentially expressed upregulated genes involved pathways of leucocyte activation, cytokine production and signaling (Figure 3A). In addition, we found the dataset enriched in BPs of the defense response against viruses and bacteria. The downregulated DEGs were significantly enriched in regulation of protein complex assembly, peptidyl serine phosphorylation and cell morphogenesis involved in differentiation (Figure 3B).

We next performed pathway analysis for these overlapping DEGs (Figure 4). Upregulated DEGs in CD4 T cells of both SRSF1 KO mice and individuals with SLE were involved in biologic processes regulating IL-10 signaling, positive regulation of cytokine signaling, leukocyte differentiation and cellular response to biotic stimulus (Figure 4a). Downregulated common DEGs from human homologues of SRSF1 KO mice and individuals with SLE identified BPs regulating the HIF1 PID pathway and regulation of protein establishment (Figure 4a). The top 5 pathways are displayed in Table 2. Amongst the differentially upregulated genes, cytokine production particularly IL-10 signaling was highlighted, while the PID-HIF1 pathway was significantly represented by the differentially downregulated genes.

In one dataset extracted from the study by Liu et al (Liu et al., 2020), individuals with SLE were grouped according to organ involvement including skin and/or renal and/or joint disease (Liu et al., 2020). This gave us an opportunity to perform pathway analysis in common DEGs between SRSF1-controlled genes and with genes segregated with organ involvement in 4 SLE (skin), 4 SLE (skin and renal disease), and 4 SLE (skin, renal and joint disease) patients (Figure 5). While most biologic processes were similar, BPs involving B cell proliferation were exclusively involved in individuals with skin and kidney disease. Regulation of innate immune pathways appeared to be involved in individuals with joint disease (Figure 5).

Identification of major protein interaction networks in SLE that may be controlled by SRSF1

Next, we performed interactome analysis of common DEGs between human homologues of upregulated genes controlled by SRSF1 and individuals with SLE to identify protein-protein interaction (PPI) networks regulated by SRSF1 (Figure 6). 151 functional interactions were identified in the upregulated DEG group with a PPI enrichment p-value of $< 1.0e\text{-}16$. Based on the information in the STRING database, the PPI network was constructed (Figure 6). We further identified top 10 genes as HUB genes with the most interactions-OAS2, IL10, IFIT3, CXCL10, CCR5, TMEM176, CSF, CCR2, CCNB2 and CCNA2 (Supplementary table 4). Additionally, using the MCODE plugin we identified clusters (highly interconnected regions) in a network. We selected the top 3 significant modules and analyzed the cellular pathways of the genes involved in these modules (Supplementary Table 5).

We performed similar analysis for the downregulated DEG cluster and found only 5 interactions with a PPI enrichment p-value of 0.338. This indicates that this set of proteins are essentially a random collection of proteins that are not very well connected through physical protein-protein interactions. Using the MCODE plugin the only module identified in this cluster was for hemostasis (Supplementary Table 5).

Identification of RNA binding targets for SRSF1 in individuals with SLE

SRSF1 is the prototype member of the serine arginine (SR) family of splicing factors (Das et al., 2013). The SR proteins have a modular domain structure with an N-terminal RNA-binding domain (RBD) and

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a C-terminal RS (arginine/serine-rich) domain which is involved in protein-protein interactions (van Der Houven Van Oordt et al., 2000). Within its RBD, SRSF1 has two RNA recognition motifs (RRM), which recognize specific RNA sequences within target genes (Aubol et al., 2018). To identify targets that may be regulated by RNA-binding of SRSF1, we evaluated the protein sequence of SRSF1 for RNA binding partners using RPINbase. The entire list of predicted hits is displayed in Supplementary table 6. We then filtered this list based on overlapping DEG in the Srsf1-KO mice and individuals with SLE. We analyzed the main pathways that were identified after enrichment analysis from upregulated DEG from SRSF KO mice and individuals with SLE that are regulated at the RNA level by SRSF1 (Figure 7A). The interactions between these are displayed in the interactome map in Figure 7A (left) and are displayed as a GO tree (right). For the downregulated overlapping DEGs, we did not find specific pathway enrichment however the genes regulated by SRSF1 at the RNA level were found to regulate mainly cell metabolic processes and signaling (Figure 7B). Overall, these data suggest that the genes identified through this analysis are likely RNA-binding targets of and regulated by SRSF1.

Evaluation of DEGs in a cohort of SLE patients with low SRSF1 levels

Since we have previously demonstrated that SRSF1 is decreased in a cohort of individuals with SLE (Moulton et al., 2013), we determined the prevalence of decreased SRSF1 levels amongst the available data. We found one cohort (Kyogoku et al., 2013) in which individuals with SLE had decreased SRSF1 levels compared to healthy controls (Figure 8A and B). We then evaluated the DEGs that were overlapping between the Srsf1-KO mice and this cohort of SLE patients with low SRSF1. These genes are listed in Supplementary Table 7. In this dataset, 290 genes were significantly (adjusted $p<0.05$) altered between patients with active SLE compared to healthy controls (Figure 8C). The top pathways represented in the DE genes in the Srsf1-ko mice were cell cycle, Th1 and Th2 differentiation, Th17 differentiation and cytokine-cytokine receptor interaction (Moulton and Tsokos, 2015). Overall, the CD4 Teffs showed an elevated T cell activation gene signature. Pathway analysis of the 290 DE genes in SLE patients identified interferon signaling, cytokine production, cytokine receptor interaction, cell migration and lysosomal clearance pathways. Overlapping genes between human and mouse transcriptomics data were analyzed. Specifically, we found 11 genes (CCR1, RHOG, ELL2, IFI16, IFIT3, OAS2, ZER1, PRKD2, RGS3, SAT1, SOCS1) to be significantly altered in active SLE patients, which were regulated by SRSF1 (Figure 8C, Table 3). Of these genes, IFI16, IFIT3 and OAS2 are associated with Type I interferon pathway (Chang et al., 2013; Sadler and Williams, 2008; Thompson et al., 2014), which is clearly known to be elevated and pathogenic in development of lupus (Crow, 2014). Hence, these comparative analyses suggest that SRSF1 controls genes, involved in T cell homeostasis, activation, cytokine regulation/signaling and differentiation, which are altered in patients with active SLE.

DISCUSSION

Low levels of SRSF1 has been associated with the severity of SLE in human patients as well as in mice model ((Katsuyama et al., 2020; Katsuyama and Moulton, 2021)). Specifically, T cell–restricted Srsf1-deficient mice develop systemic autoimmunity, lupus-nephritis, and an elevated T cell activation gene signature. In this study we attempted to evaluate if the pathways controlled by SRSF1 in lupus prone mice can be translated to the human SLE population. To this end, we compared the transcriptomic profiles controlled by SRSF1 in CD4 T cells from mice lacking SRSF1 which develop lupus like disease with the transcriptomes of CD4 T cells from patients with SLE. We found common genes and pathways between these gene sets and pathway signatures indicating that the target genes of SRSF1 are dysregulated in SLE and may be implicated in the pathogenesis of human autoimminity.

Our data indicate that a significant portion of upregulated DEGs are enriched in cytokine regulation and leucocyte activation and differentiation in SLE patients and SRSF1 KO mice (Table 2). Downregulated DEGs are primarily involved in the immune response, protein complex assembly and cell homeostasis (Table 2) (Kozyrev et al., 2012; Sharabi et al., 2018; Wu et al., 2018). These features are in accordance with the features of immune abnormalities typical of autoimmune diseases and are consistent with prior studies validating our current methodology (Chang et al., 2013; Crow, 2014; Sadler and Williams, 2008; Sharabi et al., 2018; Thompson et al., 2014; Wu et al., 2018). Based on the PPI network, the present study identified

the top 10 hub genes (Supplementary Table 4), of which IFIT3 and OAS2 are interferon-inducible genes whose encoding proteins are involved in the innate immune response to viral infection (Liu et al., 2020; Schmeisser et al., 2010), and are associated with a poor prognosis in SLE (Kyogoku et al., 2013; Liao et al., 2016; Wu et al., 2018). Chemokine and cytokine signaling has been described to be altered in SLE (Kaul et al., 2016) and our findings are consistent with this result.

Since SRSF1 is an RNA binding protein, we evaluated its binding partners amongst the differentially expressed overlapping genes in our mouse model and individuals with SLE. Pathway analysis for upregulated DE genes identified that interferon signaling including processes regulated through OAS2, cytokine regulation including IL-10 pathway regulation and chemokine signaling maybe regulated by this property of SRSF1 (Figure 7). This evaluation also identifies key genes in CD4 T cells that are regulated by SRSF1 and are aberrant in individuals with SLE (Figure 7, Supplementary Table 5). Through module analysis of the PPI network, the present study determined that the development of SLE through SRSF1 was closely associated with the chemokine binding to chemokine receptor and regulation of cytosolic calcium concentration (Supplementary 4). The overlapping downregulated genes indicated that the HIF PID pathway may be controlled by SRSF1 in CD4 T cells. Calcium flux represents a key component of signaling events that follow stimulation of lymphocytes (Gronski et al., 2009), because it directs events that determine the fate of the involved cells. Calcium signaling in systemic lupus erythematosus (SLE) lymphocytes is increased following engagement of immune receptors. In addition, HIF shifts the balance between follicular regulatory and helper T cells but also regulate metabolism, numbers of follicular helpers, and molecules they express to promote antibody production. HIF and hypoxia influence CD4+ T cell provision of effector cytokines in guidance to class switching, in part through mediation of cytokine-specific metabolic programs in the T cell help to humoral immunity (39). Interestingly NT5E encodes CD73 which is mostly expressed in B cells however in SLE, CD73 has been shown to be a marker of regulatory T cells, and its abnormal expression in Treg cells may participate in the pathogenesis of SLE (40). Among the upregulated genes, CCR1 and CCR5 were prominently featured (Table 2). Inhibition of several chemokine receptors including CCR1 have been shown to prevent lupus nephritis in lupus prone mice along with amelioration of autoimmunity in SLE mouse models (41-42). CCR5 is involved in the recruitment of inflammatory cells into tissues, and mechanisms modulating CCR5 expression and function interfere in SLE development, influencing the clinical course of the disease (43)

To further narrow down processes that are controlled by SRSF1, we evaluated common genes and pathways in SRSF1 KO mice and SLE patients with low expression of SRSF1 (Figure 8, Table 3). Overlapping genes between human and mouse transcriptomics data specifically identified 11 genes (CCR1, RHOG, ELL2, IFI16, IFIT3, OAS2, ZER1, PRKD2, RGS3, SAT1, SOCS1) to be significantly altered in active SLE patients and were regulated by SRSF1 as confirmed by our mouse RNA-seq analysis.

These findings are hypothesis generating and enhance the current findings which have been limited to mouse models. Some of these genes (PTEN, mTOR pathway S6) and proteins (inflammatory cytokines) have been validated by RT-PCR, western blot & flow cytometry in mice (Katsuyama et al., 2019); however, further confirmation and functional analysis for the molecules identified will be performed in future studies.

In conclusion, these hub genes may have various roles in the occurrence and development of the SLE, leading to damage of multiple systems in SLE. Combined with bioinformatics analysis, the current study identifies key genes and cellular pathways regulated by SRSF1, involved in aberrant T cell function in human SLE.

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Declaration of Competing Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

FIGURE LEGENDS

Figure 1. Workflow of bioinformatic comparative analysis of transcriptomics datasets.

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Schematic shows the workflow of comparative analysis of transcriptomics data of differentially expressed genes (DEG) from CD4 T cells from Srsf1-ko mice and SLE patients.

Figure 2. Differentially expressed genes (DEG) in CD4 T cells from *Srsf1*-ko mice overlap with genes aberrantly expressed in CD4 T cells from patients with SLE.

A . Graph shows numbers of DEGs in effector CD4 T cells from Srsf1-ko mice compared to control mice. **B.** Publicly available gene expression datasets from CD4 T cells from patients with SLE were curated from NCBI GEO and literature search. Graph shows cumulative numbers of DEG in SLE patients compared to healthy individuals from five datasets. **C.** Venn diagram shows numbers of overlapping genes between *Srsf1* -ko mice and SLE patients. **D.** Graph shows relationship between adjusted p value and log2fold expression of overlapping genes with $p<0.05$.

Figure 3. Top pathways and interactome represented by DEGs in Srsf1-ko mice and SLE patients reveal pathways in lymphocyte activation, cytokine signaling and interferon response.

A . and **B** . Metascape heatmap graphs show the top pathways associated with the upregulated and downregulated DEG in*Srsf1* -ko mice (**A**) and SLE patients (**B**). Network analysis on the right shows interactome represented by the DEGs.

Figure 4. SRSF1 controls genes involved in T cell cytokine production/signaling and differentiation which are aberrantly regulated in SLE.

Pathway enrichment analysis of overlapping DEGs from CD4 T cells from*Srsf1* -ko mice and SLE patients was performed in Metascape. Graphs show the top pathways represented by the DEGs that overlap between. Network analysis on the right shows interactions between DEGs and thickness of connecting lines indicates strength of interactions.

Figure 5. Molecular profiles stratified by target organ involvement in SLE patients

Pathway analysis of overlapping DEGs from one dataset (Liu et al., 2020) of SLE patients with distinct organ involvement. Image shows relevant pathways associated with DEGs genes overlapping between *Srsf1* -ko mice and SLE patients with respective SLE organ characteristics. (S = SLE with no organ involvement, SK = SLE with skin and kidney involvement, SKJ = SLE with skin, kidney and joint involvement.

Figure 6. Interactions of DEGs co-expressed with SRSF1.

Schematic shows co-expression analysis of DEGs with SRSF1 in *Homo sapiens* .

Figure 7. Interaction analysis of pathways enriched by RNA binding targets of SRSF1.

A. Pathway interactions of upregulated genes. On the right is shown the GO terms tree of upregulated genes. **B.** Table shows pathways enriched by downregulated gene targets of SRSF1.

Figure 8. Evaluation of overlapping DEGs in active SLE patients with decreased *Srsf1* levels.

A. RNA-sequencing data from one dataset (Kyogoku et. al., GSE51997) (Kyogoku et al., 2013) of active SLE patients versus healthy controls was analyzed using NCBI GEO2R for *Srsf1* mRNA expression.**B** . Graph shows significant decrease of *Srsf1* expression levels in CD4+ T cells in SLE patients (n=6) versus healthy controls (n=4) (p=0.004). **C.** Venn diagram shows numbers of overlapping genes.

Table 1. Table shows the sources of SLE datasets curated from NCBI GEO2R and literature searches.

Table 2. Table shows top five pathways represented by the overlapping DEGs between SLE patients and Srsf1-ko mice.

Table 3. Table shows 11 genes from one dataset (Kyogoku et al., 2013) and associated biological processes and protein function of each gene.

Supplementary Table 1. List of human homologues of DEGs identified in Srsf1-KO mice

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Supplementary Table 2. List of excluded mouse specific DEGs (not found in humans equivalent data)

Supplementary table 3 . Overlapping mouse and human DEGs

Supplementary Table 4. Selection and analysis of cellular pathways of the genes involved in the top 3 significant modules using MCODE

Supplementary Table 5. List of predicted targets regulated by RNA-binding of SRSF1 protein

Supplementary Table 6. List of predicted targets regulated by RNA-binding of SRSF1 protein.

Supplementary Table 7. List of the overlapping DEGs between Srsf1-KO mice and cohort of SLE patients with low SRSF1.

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