Autophagy-related genes are potential diagnostic biomarkers for dermatomyositis

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Background: Dermatomyositis (DM) is an autoimmune disease mainly diagnosed by its symptoms and a physical examination, with only some subtypes of DM showing clear molecular changes. To date, few biomarkers have been identified to assess DM progression. Autophagy-related genes have been significantly correlated with inflammation, several types of autoimmune diseases, and the immune response, but few studies have explored the role of autophagy-related genes in DM. Therefore, this study aimed to investigate the roles of autophagy-related genes in DM.

Methods: We collected three datasets of dermatomyositis-related transcriptome from the Gene Expression Omnibus (GEO) database: GSE1551, GSE46239, and GSE143323 and analyzed the differentially expressed genes (DEGs). We also conducted functional enrichment analyses with Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. To explore whether the autophagy-related genes were differentially expressed in DM compared with normal samples, we performed an intersection between the DEGs and autophagy-related genes obtained from the Human Autophagy Database (HADb, http://www.autophagy.lu/). Finally, we used selected autophagy-related genes as biomarkers for diagnosing and analyzing the correlation with immune cell infiltration.

Results: Our results showed that 143 genes were upregulated, and 14 were downregulated in the DM samples compared with healthy samples. The functional enrichment analysis revealed that these DEGs played a significant role in the type I interferon signaling pathway, cytokine activity, chemokine activity, double-stranded RNA binding, and blood microparticles. The intersection results identified *CCL2*, *CDKN1A*, *FOS*, *MYC*, and *TNFSF10* as the primary autophagy-related genes in DM. All showed significantly increased expressions in DM samples compared with healthy samples. We were also curious to investigate immune cell infiltration in DM. Our results showed that the selected autophagy-related genes significantly influenced the infiltration of multiple immune cells, such as B cells, macrophages, and natural killer cells. Finally, we assessed the diagnostic sensitivity of *CCL2*, *CDKN1A*, *FOS*, *MYC*, and *TNFSF10* for DM. The results showed the area under the curve (AUC) values of the ROC were 0.855, 0.889, 0.744, 0.826, and 0.816, respectively. The combined genes' diagnostic AUC value was 0.951.

Conclusions: *CCL2*, *CDKN1A*, *FOS*, *MYC*, and *TNFSF10* are potential diagnostic biomarkers for DM.

Keywords: Dermatomyositis; autophagy; biomarkers; bioinformatics analysis

Ann Transl Med 2022;10(4):228 | https://dx.doi.org/10.21037/atm-22-70

Introduction

Dermatomyositis (DM) is an autoimmune disease characterized by idiopathic inflammatory myopathy with distinguishing clinical features. For adult DM, the incidence rate is low, at about 1/100,000, although the incidence rate appears to be increasing over time (1). DM can be divided into several subtypes, distinguished by different clinical outcomes with varied manifestations. A detailed physical examination is fundamental for diagnosing DM patients. In addition to the physical examination, a better understanding of the critical role played by molecular alterations in DM occurrence and progression is needed. Several gene alterations have been significantly correlated with DM, such as DNA methylation, and in particular, human leukocyte antigens (HLA), histone modification, miRNA, and major histocompatibility complex (MHC) polymorphisms. Since DM is an autoimmune disease, several autoimmune antibodies, such as myositis-specific autoantibodies (MSAs), can be used to diagnose the idiopathic inflammatory myopathies in patients (2). MSAs can be diagnostic markers and may also significantly influence the disease process. The pathogenesis of DM is still unclear, and the main underlying mechanisms may include genetic alterations, environmental factors, and immune mechanisms. There is an urgent need to identify complementary or novel molecular biomarkers to understand the pathogenesis of DM more extensively, and these may also be identified as potential therapeutic targets. DM is always accompanied by inflammation, and several recent studies have suggested that the autophagy process is significantly correlated with inflammation and the immune response (3-5).

To date, only a few studies have investigated autophagy's role in DM (6-8). Girolamo *et al.* revealed that the autophagy markers LC3 and p62 were more highly expressed in immune-mediated necrotizing myopathy (IMNM) than in DM or polymyositis (PM), and they mainly functioned as regulators of inflammation (8). Shu *et al.* showed that CD3^{+} T cells were significantly decreased in PM/DM patients' peripheral blood and that autophagy may play a protective role for these patients (7). Immunoglobulin is the most commonly used therapy regimen for treating autoimmune diseases, including DM. However, the underlying mechanism of its activation remains poorly understood. Das *et al.* proved that immunoglobulin mediates the anti-inflammatory effects in peripheral blood mononuclear cells by inducing autophagy (9). Day *et al.* showed that high mobility group box protein 1 (*HMGB1*) was significantly increased in IMNM and inclusion body myositis (IBM), and amongst its fundamental biological functions it also acts as an autophagy regulator (10). Furthermore, along with gene alterations, long non-coding RNAs (lncRNAs) also play key roles in DM and autophagy. Li *et al.* indicated that differentially expressed lncRNAs were detected in DM patients and played a critical role in the autophagy process (11). These results show that autophagy may play a significant role in various myositis subtypes, including DM. However, few studies have comprehensively investigated the expression levels of autophagy-related genes, their other biological functions, or their roles in immune cell infiltration in DM. Hence, we performed this study. We present the following article in accordance with the STREGA reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-70/rc).

Methods

Raw data

We collected three datasets of dermatomyositis-related transcriptome from the Gene Expression Omnibus (GEO) database; GSE1551 contained 10 healthy samples and 13 DM samples, GSE46239 contained 4 healthy samples and 48 DM samples, and GSE143323 contained 20 healthy samples and 39 DM samples. The mRNA expression matrices of the three datasets were normalized and merged using the "sva" package in R software (https://www.r-project.org/). Finally, an mRNA expression matrix of 34 healthy samples and 100 DM samples was obtained. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Analysis of differentially expressed genes (DEGs)

The healthy and DM samples were grouped and analyzed using R software's "limma" package. A log2 fold change |logFC| >1 and false discovery rate (FDR) <0.05 were used as the criteria to identify DEGs.

Functional enrichment analysis of DEGs

We performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) functional enrichment analyses of the DEGs using the "clusterProfiler" package in R software, and the results were visualized with the "ggplot2" package.

Identification of DM autophagy-related genes

We collected the list of autophagy-related genes from the Human Autophagy Database (HADb, http://www.autophagy.lu/). The DM autophagy genes and DEGs were intersected, and the DM-related autophagy genes were identified for further analysis.

The expression levels of autophagy-related genes in DM and the ROC curve of the multi-index diagnostic model

We analyzed the expression levels of the autophagy-related genes between healthy samples and DM samples. We drew the receiver operator characteristic (ROC) curve of the expression levels of the autophagy-related genes in DM using the "pROC" package. We then used the "glm" R function to linearly combine five DM autophagy-related genes to construct a multi-index diagnostic model.

Single sample gene set enrichment analysis (ssGSEA)

We used ssGSEA to analyze the abundance of immune cells in healthy and DM patients, and 28 types of immune cells were identified (12). We compared the infiltration of immune cells in healthy and DM samples. Furthermore, we also analyzed the correlation between the abundance of immune cells and the expression levels of autophagy-related genes in DM.

Statistical analysis

All statistical analyses were completed using R software. The DEGs were analyzed with the "limma" package, with the threshold set to |logFC| >1 and FDR <0.05. The Wilcoxon non-parametric test was used to compare two groups, and the correlation analyses were conducted using Spearman's method. A P value<0.05 was considered statistically significant.

Results

Principal component analysis (PCA) of raw transcriptome data before and after data normalization: results of DEGs in DM

The PCA analysis indicated that, before normalization, the principal components of mRNA expressions in the GSE1511, GSE4639, and GSE143323 datasets were significantly different (Figure 1A). Nevertheless, the principal components of mRNA expressions in these three datasets were at the same level after standardization (Figure 1B).

Compared with healthy samples, 143 genes were upregulated, and 14 were downregulated in the DM samples. The heatmap shows the 100 top-ranked upregulated and downregulated genes in DM (Figure 1C).

Functional enrichment analysis of the DEGs in DM

The results of the GO functional enrichment analysis of the DEGs in DM indicated that response to virus, defense response to virus, type I interferon signaling pathway, cellular response to type I interferon, and response to type I interferon were the top five biological processes (BPs); collagen-containing extracellular matrix, blood microparticle, myofibril, platelet alpha granule lumen, and myosin filament were the top five cell components (CC); cytokine activity, chemokine receptor binding, chemokine activity, double-stranded RNA binding, and CCR chemokine receptor binding were the top five molecular functions (MFs) (Figure 2A).

The results of the KEGG analysis indicated that Coronavirus disease—COVID-19, Influenza A, Hepatitis C, Epstein-Barr virus infection, Viral protein interaction with cytokine and cytokine receptor, Chemokine signaling pathway, Measles, Complement and coagulation cascades, Toll–like receptor signaling pathway, and Pertussis were the top 10 enrichment KEGG pathways (Figure 2B).

Expression levels of autophagy-related genes in DM and healthy samples

Five genes were obtained from the intersection of 157 DM DEGs and 223 autophagy genes. These were chemokine (C-C motif) ligand 2 (CCL2), cyclin-dependent kinase inhibitor 1A (CDKN1A), fos proto-oncogene, AP-1 transcription factor subunit (FOS), MYC proto-oncogene, BHLH transcription factor (MYC), and TNF superfamily member 10 (TNFSF10) (Figure 3).

Compared with healthy samples, the expression levels of these five genes were higher in the DM samples (Figure 4).

Accuracy of the single- and multi-gene expression levels in the diagnosis of DM (ROC curve)

At the single gene expression level, the AUC values of the ROC curve for DM diagnosis were as follows: CCL2
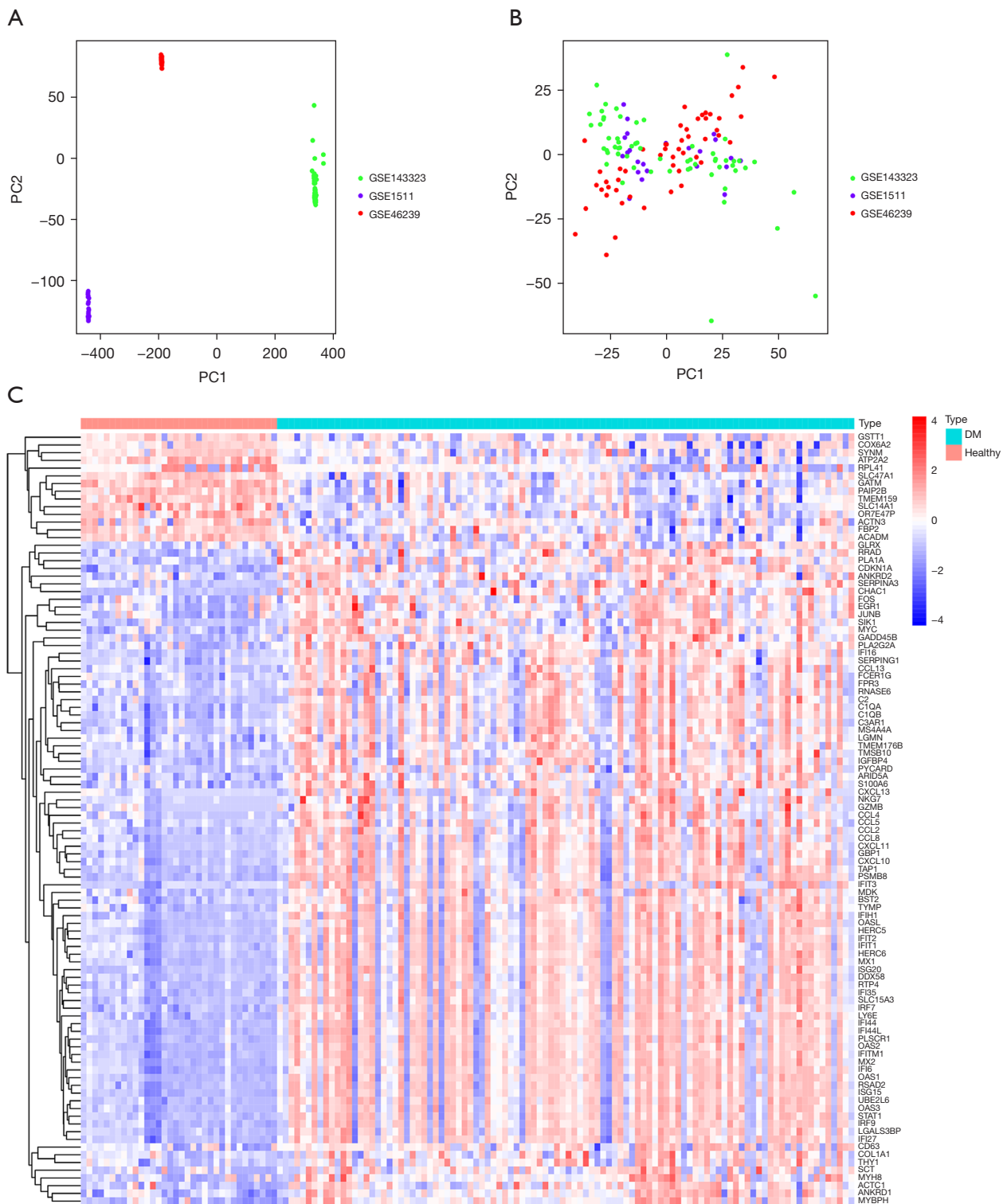
Figure 1 PCA analysis of the raw transcriptome data before and after data normalization. (A) PCA analysis before data normalization; (B) PCA analysis after data normalization. Purple represents the principal component of GSE1511, red represents the principal component of GSE46239, and green represents the principal component of GSE143323; (C) heatmap of the top 50 rank DEGs in DM. PCA, principal component analysis; DEGs, differentially expressed genes; DM, dermatomyositis.
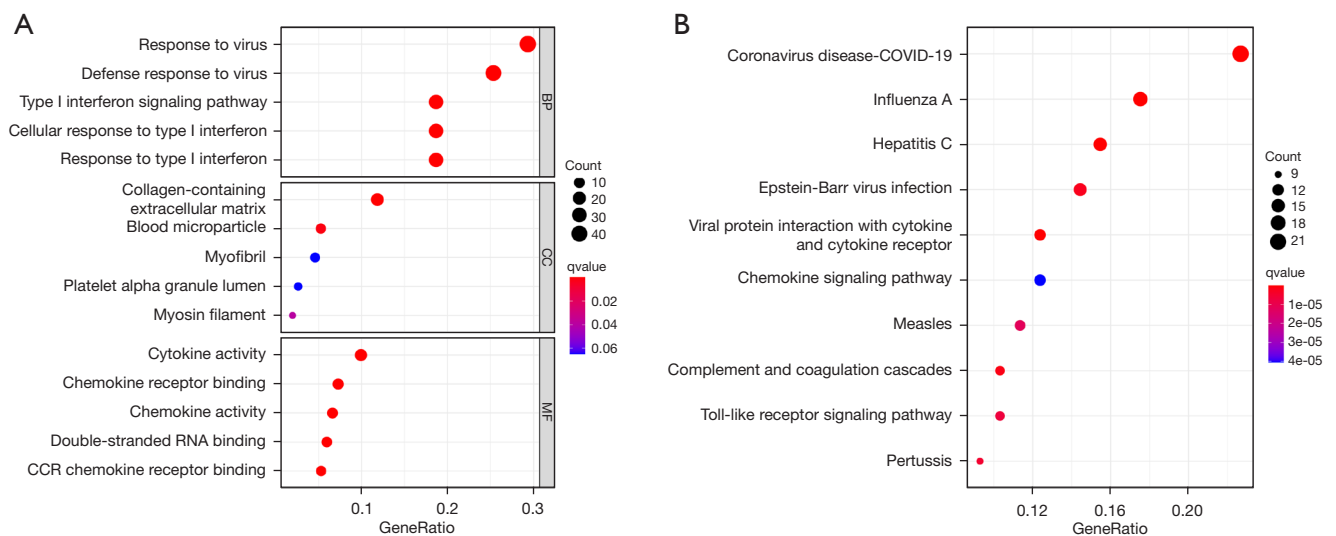
Figure 2 Functional enrichment analysis results of the DEGs in DM. (A) Bubble diagram of the GO functional enrichment analysis, including bio-logical processes, cell components, and molecular functions; (B) bubble diagram of the top 10 KEGG pathways. The bubble size represents the number of enrichment genes, and the color represents the value. DEGs, differentially expressed genes; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; DM, dermatomyositis; BP, biological process; CC, cell components; MFshid, molecular function.
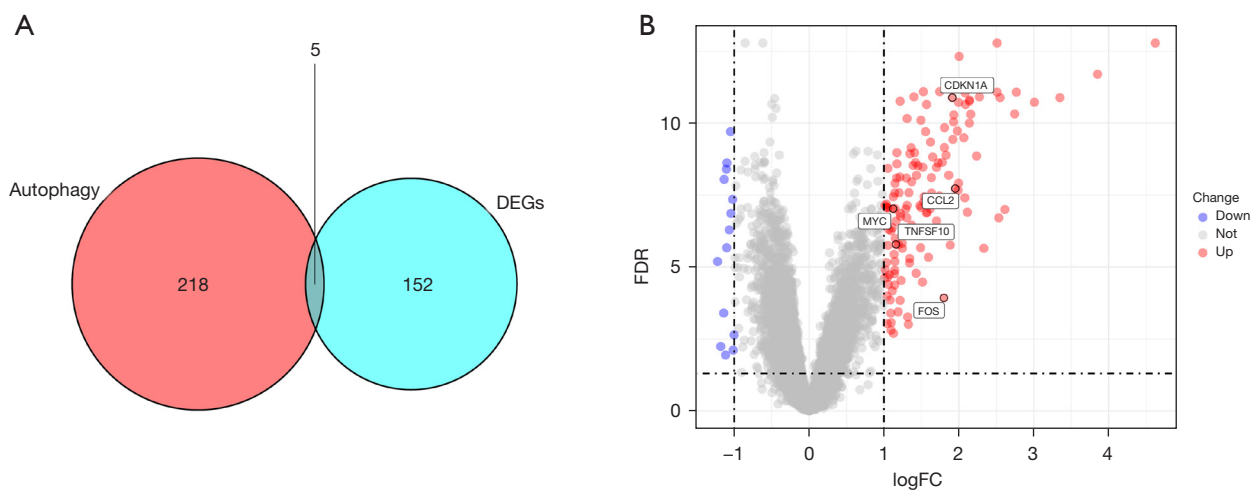
Figure 3 Intersection of DEGs and autophagy genes. (A) Venn diagram of DM DEGs and autophagy genes; (B) volcano map of the specific locations of intersecting genes in all genes. DEGs, differentially expressed genes; DM, dermatomyositis; FDR, false discovery rate.

=0.855, *CDKN1A* =0.889, *FOS* =0.744, *MYC* =0.826, and *TNFSF10* =0.816 (*Figure 5A-5E*).

After linear fitting of the multi-gene expression model (model = −19.978 + 0.769 × *CCL2* + 1.226 × *CDKN1A* + 0.487 × *FOS* + 0.235 × *MYC* + 0.460 × *TNFSF10*), the AUC value of the ROC curve of the multi-gene combined diagnosis of DM was 0.951 (*Figure 5F*).

Correlation analysis of immune cell infiltration levels and the expression levels of five DM autophagy-related genes.

Compared with healthy samples, 21 out of 28 immune cells showed high infiltration in the DM samples, including activated CD8 T cells, central memory CD8 T cells, and effector memory CD8 T cells (*Figure 6A*).
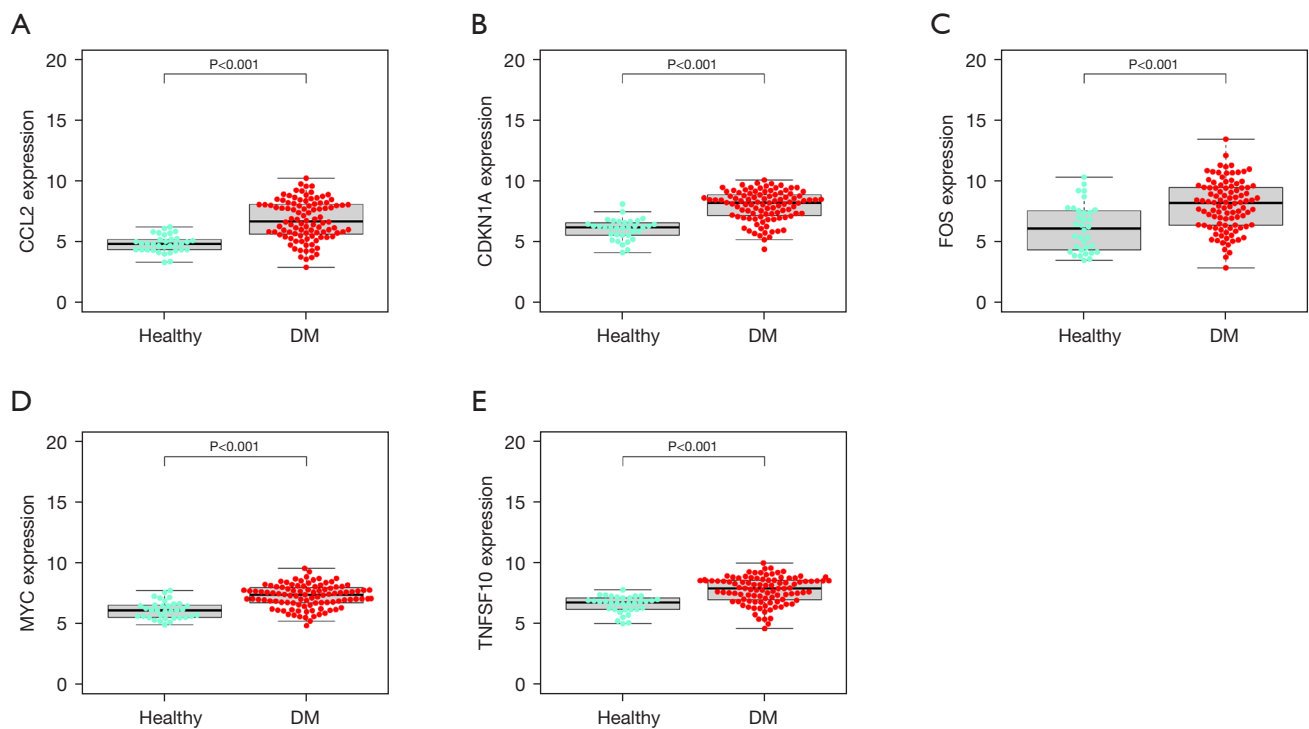
Page 6 of 11

Wang et al. Autophagy gene prediction and diagnosis of dermatomyositis

Figure 4 Expression levels of autophagy-related genes in DM and healthy samples. (A) *CCL2*; (B) *CDKN1A*; (C) *FOS*; (D) *MYC*; (E) *TNFSF10*. DM, dermatomyositis; *CCL2*, C-C motif chemokine ligand 2; *CDKN1A*, cyclin-dependent kinase inhibitor 1A; *FOS*, Fos proto-oncogene, AP-1 transcription factor subunit; *MYC*, MYC proto-oncogene, BHLH transcription factor; *TNFSF10*, TNF superfamily member 10.

The results of the correlation analysis showed a positive correlation between the expression level of five DM autophagy-related genes and the abundance of immune cell infiltration, especially *CCL2* and *TNFSF10* (*Figure 6B*).

Discussion

DM and PM are known as inflammatory myopathies and are amongst the most common autoimmune diseases. Several studies have reported on the inflammatory process and, in particular, the role of the cells (13,14). Autophagy is the process whereby subcellular components are degraded through the lysosomal signaling pathway (15). Autophagy has multiple functions in addition to the immune response and can act as the balancing factor for lymphocytes by eliminating intracellular pathogens and antigens to inhibit cell death. We know that DM is characterized by antigen presentation, immune system dysfunction, and muscle cell death and that autophagy is associated with many of these factors. However, the roles that autophagy-related genes play in DM require further exploration. Thus, we performed this study to learn whether autophagy-related genes were differentially expressed in DM than in healthy individuals. Additionally, we aimed to understand the main biological functions of these genes to identify them as complementary or novel biomarkers of the disease. By increasing our knowledge of the underlying mechanisms of autophagy-related genes in DM, there is the potential that they can assist in the diagnosis, assessment, and even treatment of DM.

The present study showed that 143 genes were upregulated and 14 were downregulated in DM samples compared with healthy samples. To understand the functions of these DEGs between the DM and healthy control groups, we conducted a functional enrichment analysis that revealed these DEGs play significant roles in various functions, including the type I interferon signaling pathway, cytokine activity, chemokine activity, CCR chemokine receptor binding, double-stranded RNA binding, and blood microparticles. Several previous studies have confirmed our finding that the type I interferon signaling pathway plays a critical role in DM (16-20). Baechler *et al.* revealed that increased expression levels of type I interferon signatures are associated with DM
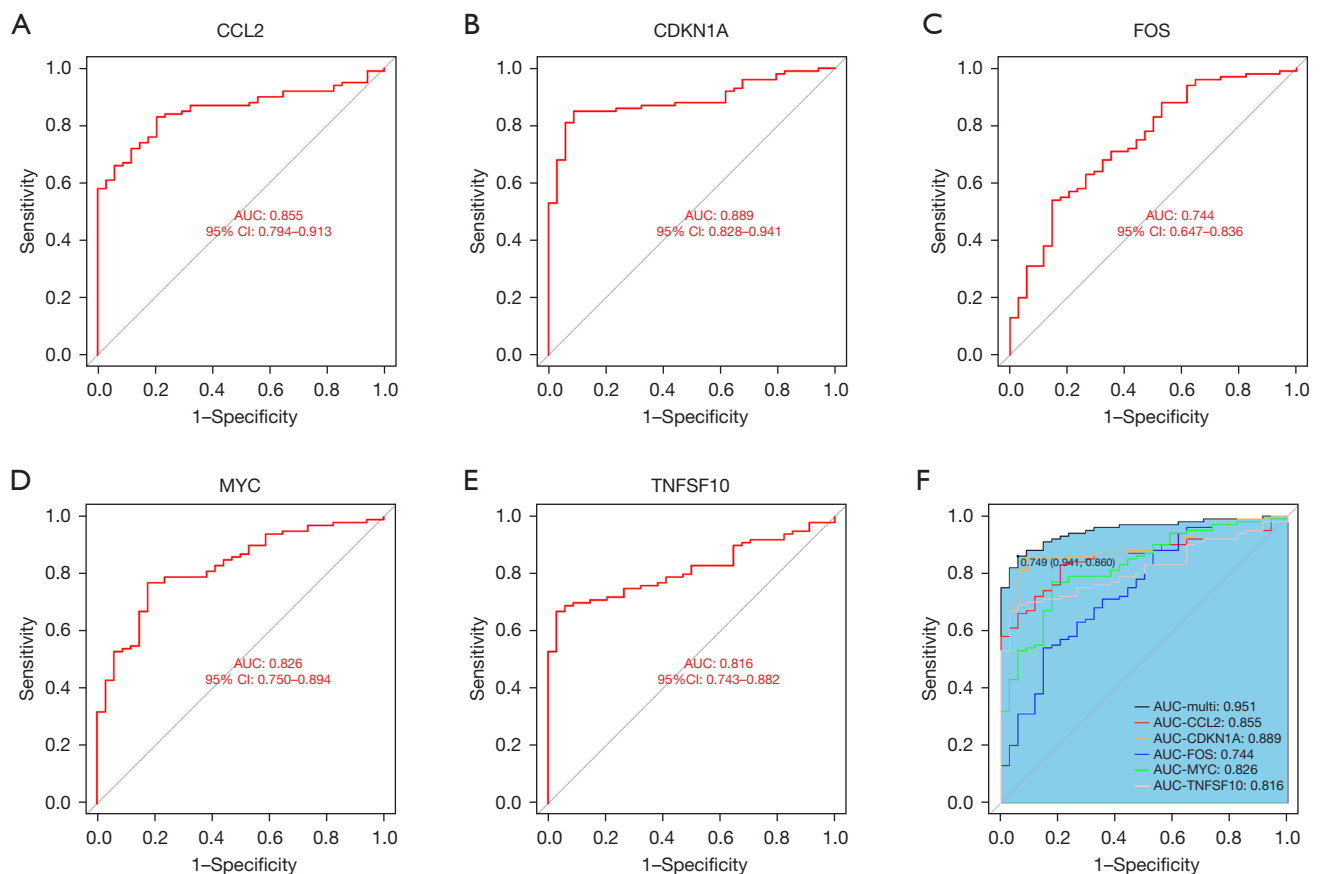
Figure 5 Accuracy of the single- and multi-gene expression levels in the diagnosis of DM (ROC curve). (A) *CCL2*; (B) *CDKN1A*; (C) *FOS*; (D) *MYC*; (E) *TNFSF10*; (F) multi-index combined diagnosis. *CCL2*, C-C motif chemokine ligand 2; *CDKN1A*, cyclin-dependent kinase inhibitor 1A; *FOS*, Fos proto-oncogene, AP-1 transcription factor subunit; *MYC*, MYC proto-oncogene, BHLH transcription factor; *TNFSF10*, TNF superfamily member 10; ROC, receiver operator characteristic; DM, dermatomyositis.

activity (16), and Gitiaux *et al.* reported type I interferon changes in juvenile DM (17). The type I interferon signature was also found to be highly expressed in the MDA5+ DM subtype (19). Based on these findings of type I interferon signaling pathway changes as critical events in DM pathogenesis, researchers have explored the efficacy of type I interferon inhibitors in the clinical treatment of DM. Ladislau *et al.* showed that JAK inhibitors improved symptoms and reduced type I interferon levels in DM patients (18). In addition to the interferon signaling pathway, other cytokines may have individual or multiple influences on DM (21). Moneta *et al.* showed that tumor necrosis factor (TNF) expression was significantly increased in DM patients prior to glucocorticoid therapy (22). Interleukin-6 (*IL-6*), interleukin-17A (*IL-17A*), and interleukin-15 (*IL-15*) have also been implicated in DM

progression (23-25). *IL-15* has been identified as a crucial biomarker for predicting the development of rapidly progressive interstitial lung disease in DM/PM patients (25). These findings show that cytokine and type I interferon signaling pathway changes are key events in the onset and progression of DM.

Given the critical role of autophagy in DM and its regulation by autophagy-related genes, we performed an intersection between DEGs and autophagy-related genes and selected the intersected genes for further analysis. The results showed that *CCL2*, *CDKN1A*, *FOS*, *MYC*, and *TNFSF10* were implicated. All exhibited significantly higher expressions in DM patients than healthy samples. Unfortunately, few studies have investigated the role of these genes in DM. Oda *et al.* found that DM patients with interstitial pneumonia (IP) who died had significantly higher
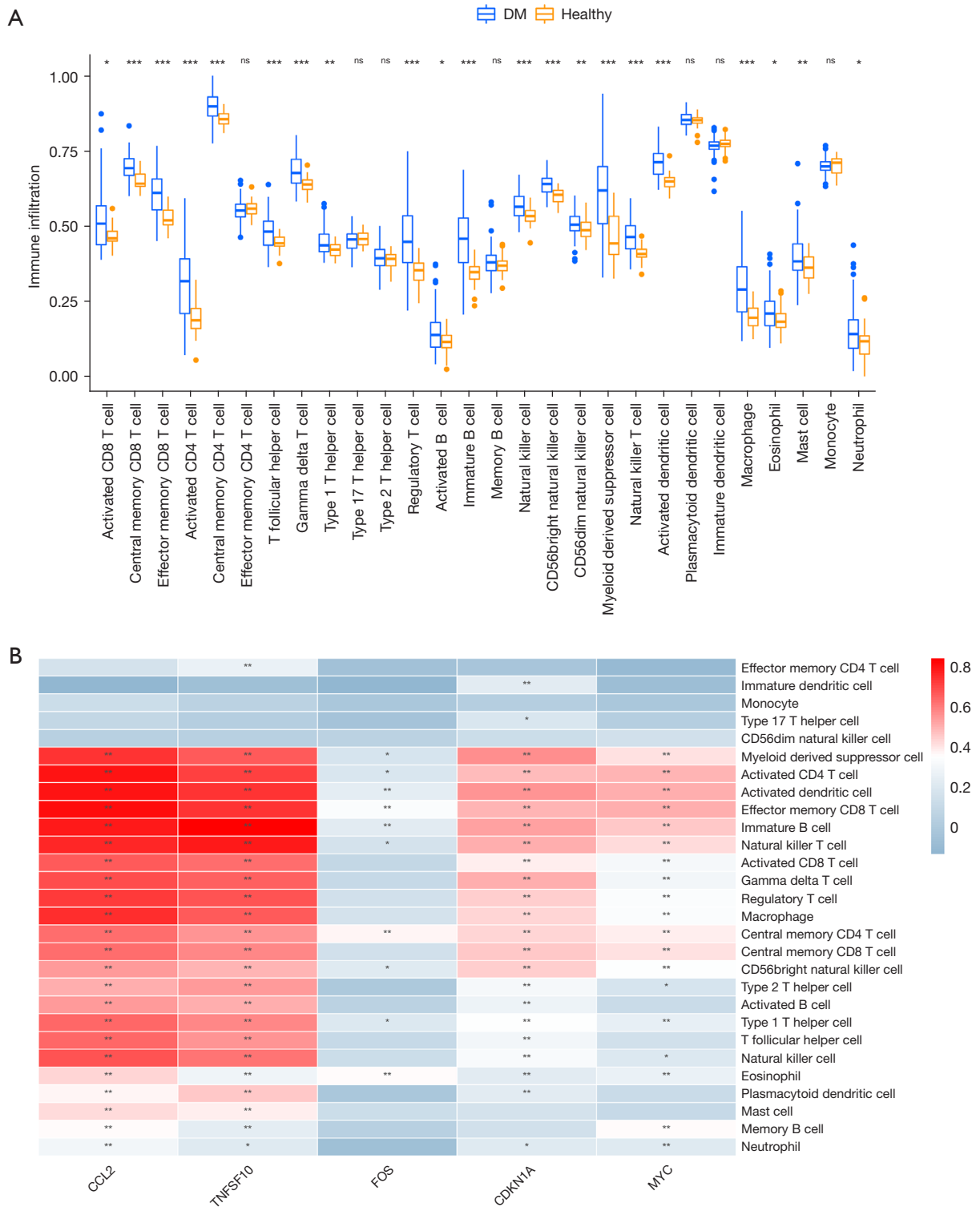
Page 8 of 11

Wang et al. Autophagy gene prediction and diagnosis of dermatomyositis

Figure 6 Correlation analysis of immune cell infiltration and the expression levels of five DM autophagy-related genes. (A) Boxplot of differential analysis of immune cell infiltration levels in healthy and DM samples; (B) heatmap of immune cell infiltration levels and DM autophagy-related genes, where red represents a strong positive correlation, and gray represents a weak positive correlation. $^{ns}P>0.05$, *$P<0.05$, **$P<0.01$, ***$P<0.001$. DM, dermatomyositis.

CCL2 serum levels than those who survived (26). This result indicates that CCL2 is not only significantly associated with DM occurrence but also with its progression. *CCL2* has also been implicated in systemic sclerosis (27). To date, CDKN1A/p21 has not been investigated in DM. Seleznik *et al.* reported that *CDKN1A* promotes inflammation in autoimmune pancreatitis (28). Aljabban *et al.* indicated that *MYC* was associated with interferon signaling pathway changes and was upregulated in DM patients (29). *TNFSF10* is a critical inflammation regulator and has been shown to play a key role in several diseases associated with inflammatory changes, including various cancers and Alzheimer's disease (30-32). The above results confirm that autophagy plays a significant role in DM and other inflammatory diseases.

The functional enrichment analysis and the intersection of autophagy-related genes showed that their biological function was enriched in the inflammation and cytokine signaling pathways. Given that immune cells play an essential role in the inflammation process, we were curious to investigate immune cell infiltration in DM patients. Our results showed that the final selection of autophagy-related genes significantly influenced the infiltration of multiple immune cells. Immune system changes are the primary underlying factor in autoimmune diseases, and immune cell dysregulation has been observed in various autoimmune diseases, such as systemic lupus erythematosus (SLE), Behçet's disease, and MPO-ANCA vasculitis (33-35). However, few studies have clarified the role of immune cells in DM. Cassius *et al.* reported T cell dysregulation in patients with active DM (36), and B cells, macrophages, and natural killer cells have also been shown to significantly affect DM progression (37-39). This study identified *CCL2*, *CDKN1A*, *FOS*, *MYC*, and *TNFSF10* as potential biomarkers for DM. Shu et al found that autophagy may serve a potential protective role in the peripheral blood T cells of patients with DM (7). Our results show that activated CD8 T cells, central memory CD8 T cells and effector memory CD8 T cells were high infiltration in the DM samples, therefore, *CCL2*, *CDKN1A*, *FOS*, *MYC*, and *TNFSF10* of hub autophagy genes may treat DM by regulating immune cells. However, the limitation of our study is the lack of expression and functional investigation of hub autophagy genes.

Conclusions

The autophagy-related genes *CCL2*, *CDKN1A*, *FOS*, *MYC*,

and *TNFSF10* showed significantly higher expressions in DM samples than healthy samples, and their most important biological functions include cytokine and type I interferon signaling pathway regulations. Additionally, they have a significant influence on immune cell infiltration in DM. They may also serve as potential biomarkers for diagnosing DM.

Acknowledgments

Footnote

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Page 10 of 11

Wang et al. Autophagy gene prediction and diagnosis of dermatomyositis

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(English Language Editor: D. Fitzgerald)