Research article

Investigating genetic drivers of dermatomyositis pathogenesis using meta-analysis

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ABSTRACT

Aims: Dermatomyositis (DM) is a progressive, idiopathic inflammatory myopathy with poorly understood pathogenesis. A hallmark of DM is an increased risk for developing breast, ovarian, and lung cancer. Since autoantibodies against anti-TIF-1-γ, a member of the tripartite motif (TRIM) proteins, has a strong association with malignancy, we examined expression of the TRIM gene family to identify pathways that may be contributing to DM pathogenesis.

Materials and methods: We employed the Search Tag Analyze Resource for GEO platform to search the NCBI Gene Expression Omnibus to elucidate TRIM family gene expression as well as oncogenic drivers in DM pathology. We conducted meta-analysis of the data from human skin (60 DM vs 34 healthy) and muscle (71 DM vs 22 healthy).

Key findings: We identified genes involved in innate immunity, antigen presentation, metabolism, and other cellular processes as facilitators of DM disease activity and confirmed previous observations regarding the presence of a robust interferon signature. Moreover, analysis of DM muscle samples revealed robust upregulation of TRIM14, TRIM22, TRIM25, TRIM27, and TRIM38. Likewise, analysis of DM skin samples showed upregulation of TRIM5, TRIM6, TRIM 14, TRIM21, TRIM34, and TRIM38 and downregulation of TRIM73. Additionally, we noted upregulation of oncogenes IGLC1, IFI44, POSTN, MYC, NPM1, and IDO1 and related this change to interferon signaling. While the clinical data associated with genetic data that was analyzed did not contain clinical data regarding malignancy in these cohorts, the observed genetic changes may be associated with homeostatic and signaling changes that relate to the increased risk in malignancy in DM.

Significance: Our results implicate previously unknown genes as potential drivers of DM pathology and suggest certain TRIM family members may have disease-specific roles with potential diagnostic and therapeutic implications.

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1. Introduction

Dermatomyositis (DM) is an idiopathic inflammatory myopathy characterized by progressive, autoimmune inflammatory changes primarily involving the skin and skeletal muscles. Clinical cutaneous manifestations include Gottron's papules and sign, shawl and V-signs as well as heliotrope rash [1]. Inflammation of the skeletal muscle results in painless, proximal muscle weakness. Histopathologically, DM muscle biopsies are characterized by perivascular and perimysial inflammation with a predominance of CD4+ T cells, perifascicular atrophy and the presence of terminal complement C5b–9 membrane attack complex [2]. Recent data suggest that sarcoplasmic myxovirus resistance A (MxA) expression in muscles by immunohistochemistry is a specific marker for DM and better than conventional hallmarks [3, 4, 5]. Interestingly, complement proteins can be detected in DM muscles before inflammatory cell infiltration [6]. The presence of myositis-specific and myositis-associated antibodies have varying clinical significance and it is unclear what role they play in pathogenicity [1, 7]. For example, anti-Mi-2 is considered a marker for steroid-responsive acute DM [7]. Likewise, anti-MDA-5 is associated with amyopathic DM, anti-TIF-1-γ/α is associated with increased risk of malignancy, and anti-NXP-2 with both malignancy-associated and juvenile DM with calcinosis [7]. Risk of malignancy is higher in DM compared to other idiopathic myopathies and is typically associated with carcinomas of the ovary, breast, lung, GI tract, and non-Hodgkin's lymphoma [1, 8, 9]. The exact type of cancer in DM patients is a function of origin, age, and gender [10, 11]. However, the pathogenesis underlying DM-associated malignancy risk remains poorly understood. Current evidence points to changes in cellular and humoral immunity [10]. Another cause could be cross-reaction to self-antigens which may allow cancer cells to evade the immune response. Indeed, myositis related auto-antigens such as histidyl-t-RNA synthetase, the target autoantigen in the anti-synthetase syndrome, have been found in regenerative muscles alongside other malignancies including breast cancer, hepatocellular carcinoma, and lung cancer [12, 13].

The etiology of adult DM is thought to involve both genetic and environmental influences from HLA alleles and viral triggers, respectively, but a concise pathogenetic process remains unknown [7]. Classically, the etiology of DM was believed to be humorally-mediated due to the presence of B-cells, CD4+ T cells, macrophages and plasma cells within muscle tissue [9]. However, recent evidence suggests DM may be mediated via type 1 interferon, suggesting a synergistic activity of a dysregulated innate and adaptive immune response [4, 9, 14, 15, 16]. Another potential etiology of DM may come from humorally-mediated effects on the Tripartite Motif (TRIM) family of proteins. The large family of TRIM proteins play important roles in a variety of cellular functions including differentiation and apoptosis as well as regulating the host antiviral response [17, 18]. For example, TRIM22 is known to modify HIV replication while TRIM19 limits replication of a myriad of RNA and DNA viruses [19, 20]. TRIM proteins are also involved in sarcolemmal resealing and repair responses. TRIM72 (also known as MG53) is a necessary component of the sarcolemmal repair response in striated muscle, deficiency of which results in significant myopathy and susceptibility to cardiovascular injury in mice [21, 22, 23, 24, 25, 26]. Lastly, auto-antibodies to TRIM proteins themselves have been linked to idiopathic inflammatory myopathies (IIM), including to DM [27, 28, 29, 30]. TRIM proteins may contribute to DM pathology in several ways including coordination of a maladaptive immune response, generation of cross-reactive autoantibodies, or other undiscovered mechanisms. Moreover, a recent publication demonstrated that some DM patient have antibodies to TRIM72 that are capable of compromising cell membrane repair that allowing for aberrant leakage of cell content that could promote autoimmunity [31]. Given the putative role of TRIM proteins in DM, application of a bioinformatics approach examining the role of these mechanisms may provide crucial insight into the pathogenesis of DM and facilitate the identification of novel therapeutic targets.

2. Methods

The National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) is an open database containing millions of biological samples from functional genomics experiments. The Search Tag Analyze Resource for GEO (STARGEO) platform allows for meta-analysis of genomic signatures between normal and diseased tissue through tagging of biological samples from public experiments. More information on STARGEO and can be found in our previous paper [32]. We conducted two meta-analyses on adult human skin (60 DM vs 34 healthy) and muscle biopsy (71 DM vs 22 healthy) samples. DM here is defined by clinical and histopathological criteria [33, 34, 35, 36]. Briefly, to be diagnosed with definitive DM patients had to meet inclusion criteria of onset after 18 and an insidious or subacute onset, pattern of weakness (symmetric proximal > distal and neck flexor > extensor), and rashes typical of DM including Gottron's sign, V-sign, shawl sign, and heliotrope periorbital edema. Patients had to also demonstrate elevated serum creatinine kinase and electromyography consistent with DM. Electromyography had to display motor unit action potential (MUAP) durations that were short in duration, polyphasic, and small in amplitude. Increased spontaneous activity also had to be seen in the form of fibrillation potentials and positive sharp waves. Lastly, muscle biopsies had to include the following features: endomysial T cell infiltrates surrounding and invading non-necrotic fibers, perifascicular atrophy, perivascular and perimysial inflammatory cell infiltrate, MAC depositions on small blood vessels or reduced capillary density, and endomysial CD8 T cells surrounding non-necrotic fibers.

DM muscle samples were taken from series GSE1551, GSE 3307, and GSE48280 and DM skin samples were taken from series GSE32245 and GSE46239. As these studies do not define DM subtypes nor provide myositis-specific autoantibody presence, we combined samples across studies to elucidate general mechanisms of pathogenesis [14, 16, 37, 38, 39]. Meta-analysis was also conducted in rheumatoid arthritis (RA) blood (114 RA vs 90 healthy; samples taken from series GSE15573 and GSE17755) and synovial fluid (130 RA vs 68 healthy; samples taken from series GSE12021, GSE13026, GSE1919, GSE2053, GSE21959, GSE29746, GSE55235, GSE55457, and GSE77298), sarcoidosis (SD) bronchoalveolar lavage (21 SD vs 20 healthy; samples taken from series GSE75023 and GSE110779) and peripheral blood (216 SD vs 271 healthy; samples taken from GSE18781, GSE1907, GSE19314, GSE37912, GSE42825, GSE42826, GSE42830, GSE42832, and GSE83456), systemic sclerosis (SSc) skin (63 SSc vs 31 healthy; samples taken from GSE12493 and GSE9285), and Kawasaki (KA) peripheral blood (121 KA vs 40 healthy; samples taken from GSE18606, GSE68004, and GSE109351) for comparative TRIM activity analysis. We were able to extract large number of genes for each of the meta-analyses conducted using STARGEO (see Table 1). We analyzed gene signature outputs with Ingenuity Pathway Analysis (IPA), restricting genes showing statistical significance (p < 0.05) and an absolute experimental log ratio greater than 0.1 between case and control samples. A total of 1,392 and 461 genes from DM muscle and skin analysis, respectively, were included in the IPA analysis. These selected genes were used for the next step analysis in IPA to elucidate biological process, disease mechanisms, and potential biomarkers and therapeutic targets that will be highlighted in the results and discussion sections. Table 1 details top up and downregulated genes for the DM skin and muscle analyses. See supplemental tables 1 and 2 for a complete list of gene p-values and experimental log ratios.

IPA allows for robust analysis of omics data using current knowledge of disease processes, biological pathways, and activity of existing drugs. The relationship between genes, disease processes, phenotype, and drug activity can be searched for and highlighted in inputted genetic studies. IPA contains a myriad of facts from genomic experiments of many modalities and allows us to dissect complex biological networks that characterize genomic, metabolomic, and proteomic data [40]. IPA enables us to take full advantage of our approach's novel use of large-scale data, and results from IPA analysis are demonstrated in this paper.

Table 1. Summary of the top up and down-regulated genes from the meta-analysis of muscle and skin samples from dermatomyositis patients. Experimental log ratios indicating magnitude of change from control samples are shown.

Dermatomyositis Muscle				Dermatomyositis Skin			
Top Upregulated		Top Downregulated		Top Upregulated		Top Downregulated	
IGHG3	4.354	OR7E47P	-2.049	CXCL10	0.657	GAL	-0.475
GBP1	4.028	SSX2/2B	-1.541	IFI44	0.656	DDX3Y	-0.460
RSAD2	3.654	HKDC1	-1.360	ISG15	0.622	FADS1	-0.459
IFIT3	3.512	C2orf72	-1.313	KLRC1	0.545	RPS4Y1	-0.421
IGLC1	2.624	ZNF710-AS1	-1.261	RSAD2	0.536	GLDC	-0.399
IFIT5	2.496	RASA4	-1.250	OAS2	0.528	RPS4Y2	-0.397
IFI44	2.398	LOC100996756	-1.244	MX2	0.496	KDM5D	-0.346
IFIT2	2.338	AMACR	-1.233	BST2	0.495	PM20D1	-0.292
STAT1	2.272	TFRC	-1.207	OASL	0.493	EIF1AY	-0.292
POSTN	2.152	GNB1L	-1.147	HERC5	0.479	PDE6A	-0.288

All data analyzed were taken from Gene Expression Omnibus [41, 42]. There was no interaction or intervention with human subjects and no involvement with access to identifiable private patient information. As such, no IRB approval was necessary.

3. Results

3.1. Skin sample analysis

Ingenuity Pathway Analysis (IPA) demonstrates the top canonical pathways mediating the cutaneous inflammatory response in DM include interferon (IFN) signaling ($p = 1.97E-15$; Figure 1), role of pattern recognition receptors in recognition of bacteria and viruses ($p = 7.04E-06$), and activation of interferon regulatory factors (IRF) by cytosolic pattern recognition receptors ($p = 1.83E-05$). Upstream regulators include interferon regulatory factor 7 (IRF7, $p = 3.82E-57$), IFNα ($p = 2.56E-55$, Figure 2), IFNα2 ($p = 2.89E-47$), IFNγ ($p = 7.21E-46$), and STAT1 ($p = 2.30E-49$). All top upstream regulators demonstrated predicted activation. Our meta-analysis yielded thousands of strongly up and downregulated genes with various implications in pathology including inflammation, antigen presentation, metabolism, and other cellular processes.

The top upregulated molecules include chemokines such as CXCL10 and interferon-induced genes including IFI44, viperin or RSAD2, interferon-induced GTP-binding protein MX2, bone marrow stromal antigen BST2, and the tryptophanyl-tRNA synthetase WARS, and many others. Other top upregulated genes are implicated in antiviral activity and include OAS2 and OASL, antiviral enzyme HERC5, and killer cell lectin-like receptor KLRC1. Additionally, we noted stark upregulation of the antigen processing gene TAP2 and apolipoprotein L6, or APOL6.
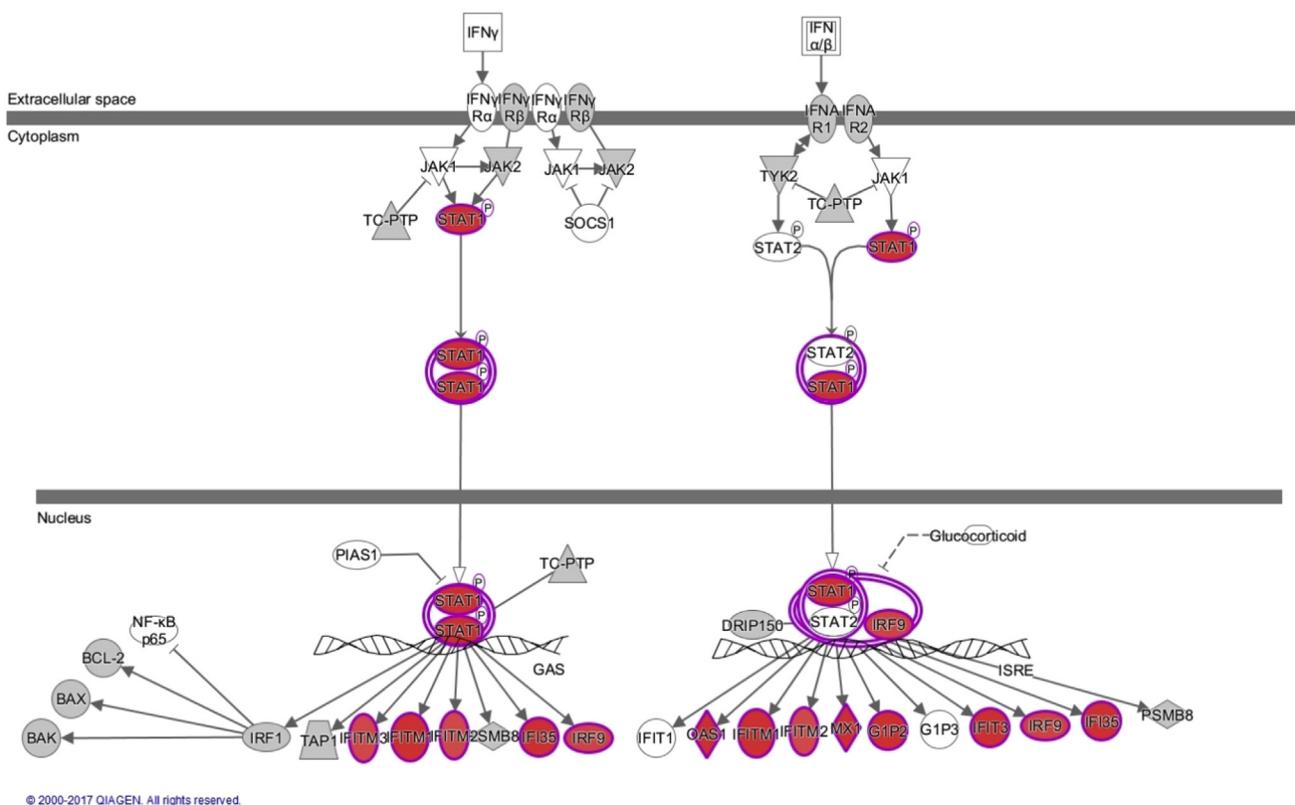
Figure 1. Interferon signaling from dermatomyositis skin analysis. Canonical pathway identified and illustrated by Ingenuity Pathway Analysis. Genes highlighted in red are upregulated from the DM skin meta-analysis, hue indicates magnitude of upregulation.
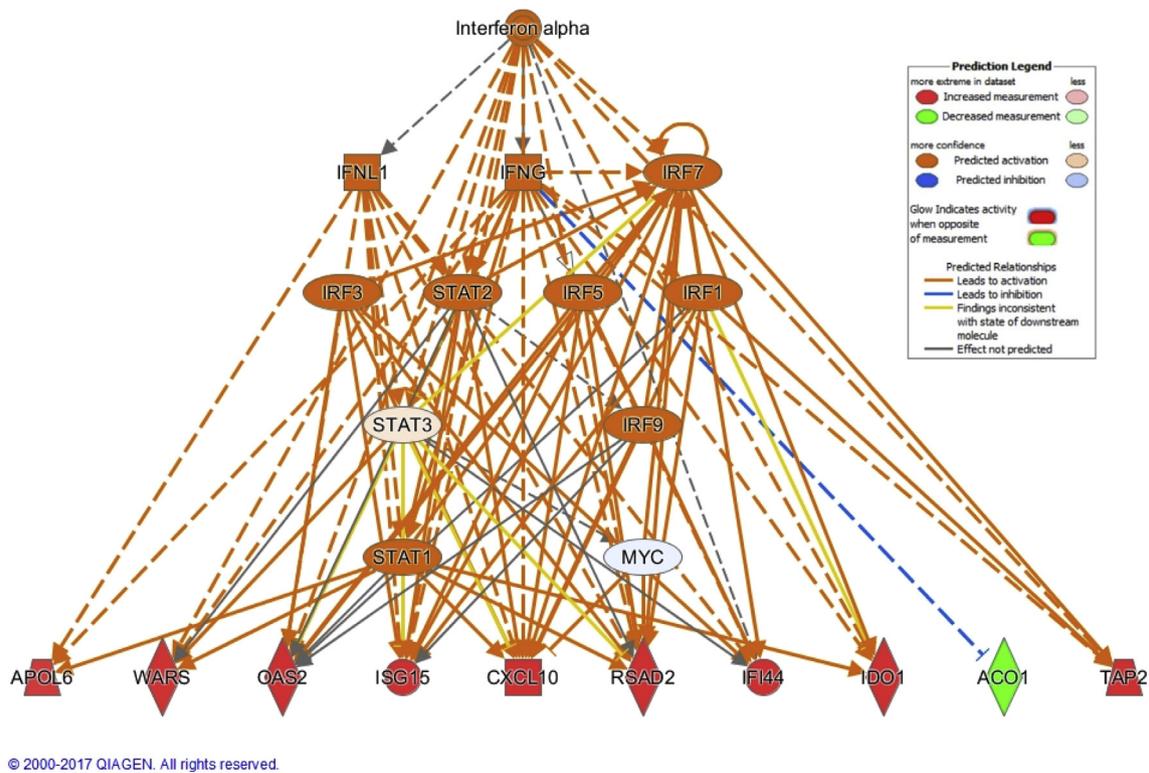
Figure 2. Ingenuity Pathway Analysis of candidate genes downstream of interferon-alpha signaling from dermatomyositis skin analysis. Genes are implicated in several disease potential disease processes including inflammation, tumorigenesis, antigen presentation, and metabolism. Legend illustrates relationship between genes.

Top downregulated genes were largely involved in metabolism, protein regulation and translation. The top downregulated genes are the regulatory peptide galanin or GAL, the putative RNA helicase DDX3Y, and the cytoplasmic ribosomes RPS4Y1 and RPS4Y2. Other top down-regulate genes have various metabolic roles and include glycine dehydrogenase GLDC, lysine demethylase KDM5D, lipid enzyme and lipid regulator PM20D1, and the aconitase ACO1. Lastly, given type 1 IFNs are well-studied drivers of DM pathogenesis, we investigated the relationship of IFNα activity with genes highlighted above (Figure 2).

3.2. Muscle sample analysis

IPA demonstrates top canonical pathways identified by muscle biopsy analysis include the antigen presentation pathway (p = 6.98E-14, Figure 3), communication between innate and adaptive immune cells (p = 2.20E-7), Type 1 Diabetes Mellitus (p = 4.47E-7), dendritic cell maturation (p = 4.90E-7), and Tec kinase signaling (p = 8.44E-7). Upstream regulators are IFNα2 (p = 1.45E-27), IFNγ1 (p = 2.22E-25), IFNα (p = 6.16E-25; Figure 4), and IFNγ (p = 8.42E-21), all with predicted activation. MAPK1 (p = 2.69E-18) is also a top upstream regulator and showed predicted inhibition of activity. Top up and downregulated genes implicate IFN signaling, tumorigenesis, metabolism, and other disease processes as potential drivers of DM pathogenesis.
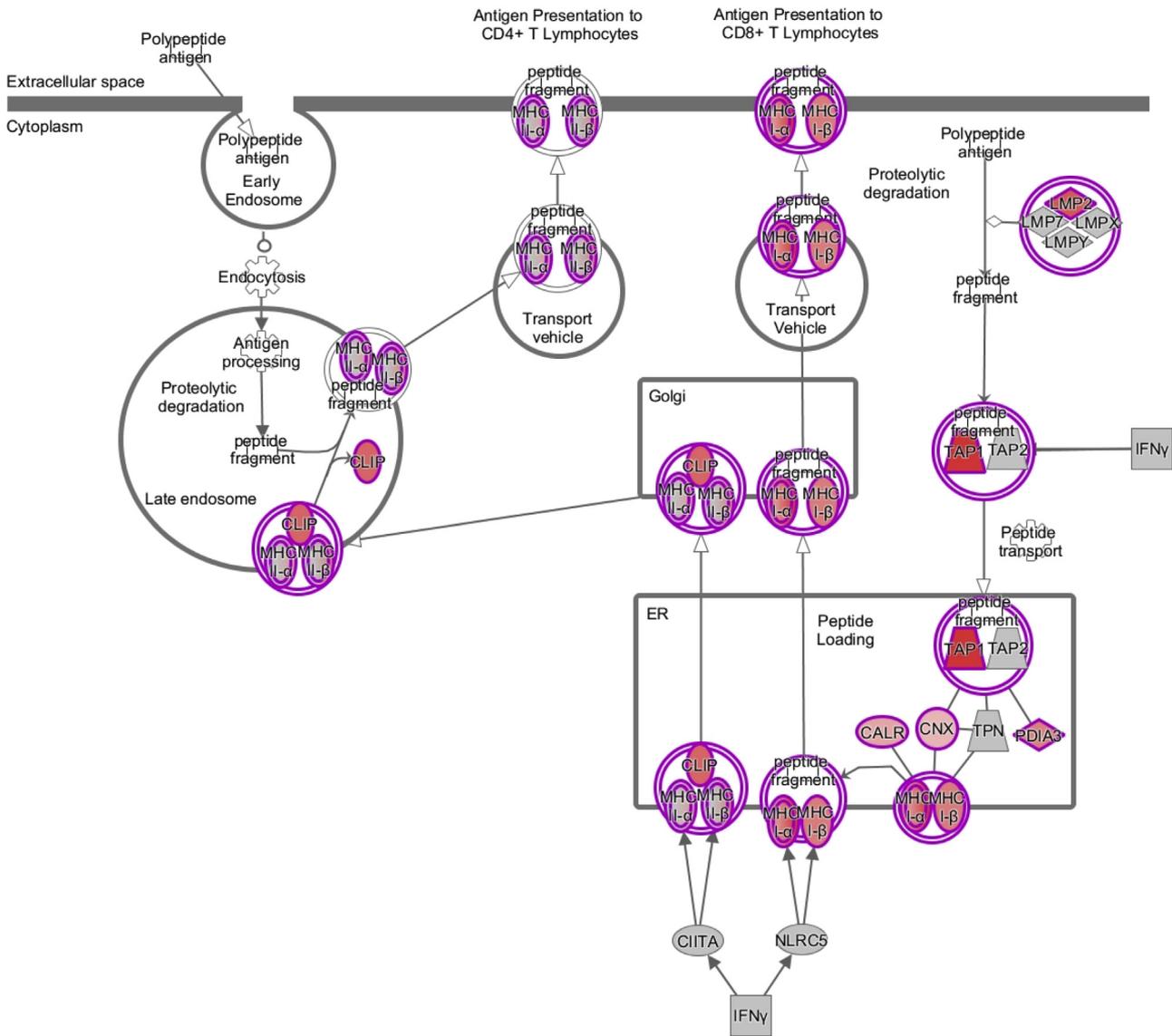
Top upregulated genes in our muscle analysis largely featured interferon-inducible genes. Among them are IFIT3, IFIT5, IFI44, and IFIT2. There is also strong upregulation of RSAD2 mentioned above and the transcription factor STAT1. We also found genes involved in cellular adhesion including the integrin ligand periostin or POSTN and the innexin pannexin 1 or PANX5. Other top upregulated genes are implicated in innate immune function and antigen processing/regulation and include DDX58 or RIG-I, the serum complement subcomponent C1QB, the pattern recognition receptor TLR3, immunoglobin IGHG3, and various HLA genes such as HLA-A, HLA-C, and HLA-DRB1.

Several identified downregulated genes have metabolic activity and include hexokinase or HKDC1, transferrin receptor (TFRC), glycine hydroxymethyltransferase (SHMT2), APOC2, enolase 3 or ENO3, cytochrome c oxidase, fumarylacetoacetate hydrolase or FAH, ferroxidase or FXN, and transferrin or TFRC. Other downregulated genes include GAP1 family member RASA4, transcription repressors SSX2, and pseudogene OR7E47P. Additionally, given type 1 IFNs are well described drivers of DM pathogenesis, we investigated the relationship of IFNα activity with genes highlighted above (Figure 4).

Lastly, we investigated tumorigenic gene expression in DM muscle samples and found expression of several novel gene candidates. We found upregulation of the MYC proto-oncogene, CD44, NPM1, immunoglobulin lambda constant 1or IGLC1, IFI44, POSTN, and indoleamine 2,3-dioxygenase 1 or IDO1. While IFNγ has known anti-tumor activity, it also promotes tumorigenesis [43]. Given this, we next investigated the relationship between the tumorigenic genes above and IFNγ signaling (Figure 5). Moreover, IPA identified cell survival, morphology, and RNA processing as one of the top networks in our DM muscle analysis (Figure 6).

3.3. Investigation of TRIM gene expression

TRIM protein biology is an active area of investigation in multiple disease states. As discussed previously, various TRIM proteins are involved in innate immunity and membrane repair, to name a few. From our DM skin biopsy analysis, we found upregulation of TRIM genes TRIM5, TRIM14, TRIM34, TRIM6, TRIM21, and TRIM38. TRIM73 was the only downregulated TRIM gene. From our muscle biopsy analysis, we found upregulation of TRIM14, TRIM22, TRIM25, TRIM27, and TRIM38. Our group recently published protein expression data demonstrating that TRIM 25 and 72 are upregulated in muscle tissue from patients with DM [31]. Given immunomodulatory activity of various TRIM members, we illustrated the relationship of the TRIM genes above with IFNγ signaling

Figure 3. Antigen presentation from dermatomyositis muscle analysis. Canonical pathway identified and illustrated by Ingenuity Pathway Analysis. Genes highlighted in red are upregulated genes from the DM muscle meta-analysis, hue indicates magnitude of upregulation.
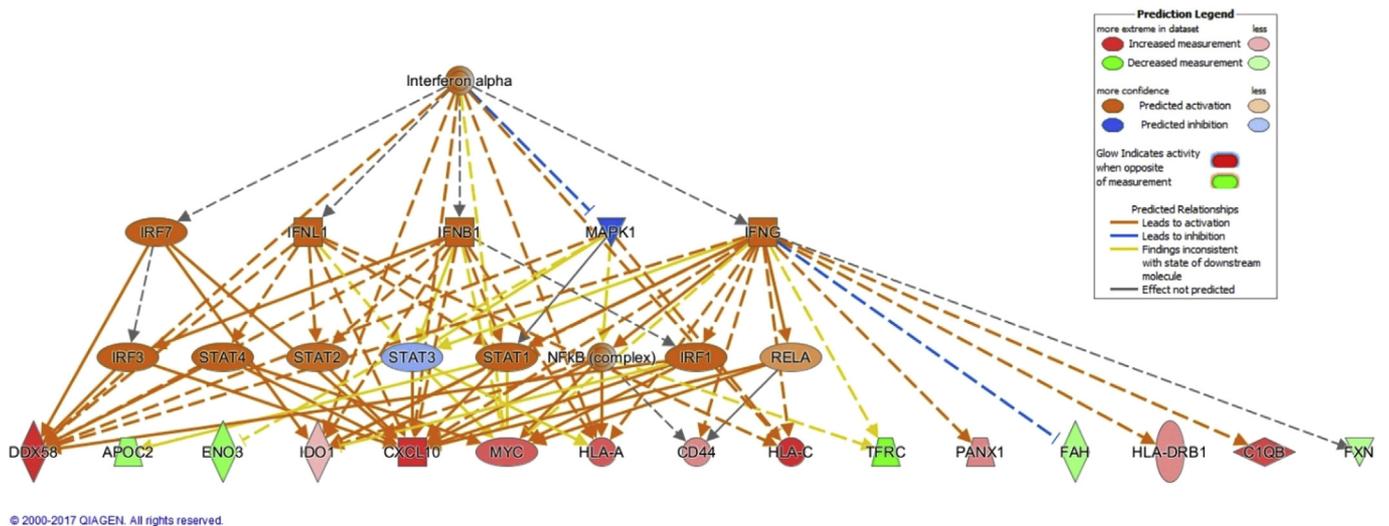
Figure 4. Ingenuity Pathway Analysis of candidate genes downstream of interferon-alpha signaling from dermatomyositis muscle analysis. Genes are implicated in several disease processes including inflammation, tumorigenesis, antigen presentation, and metabolism. Legend illustrates relationship between genes.
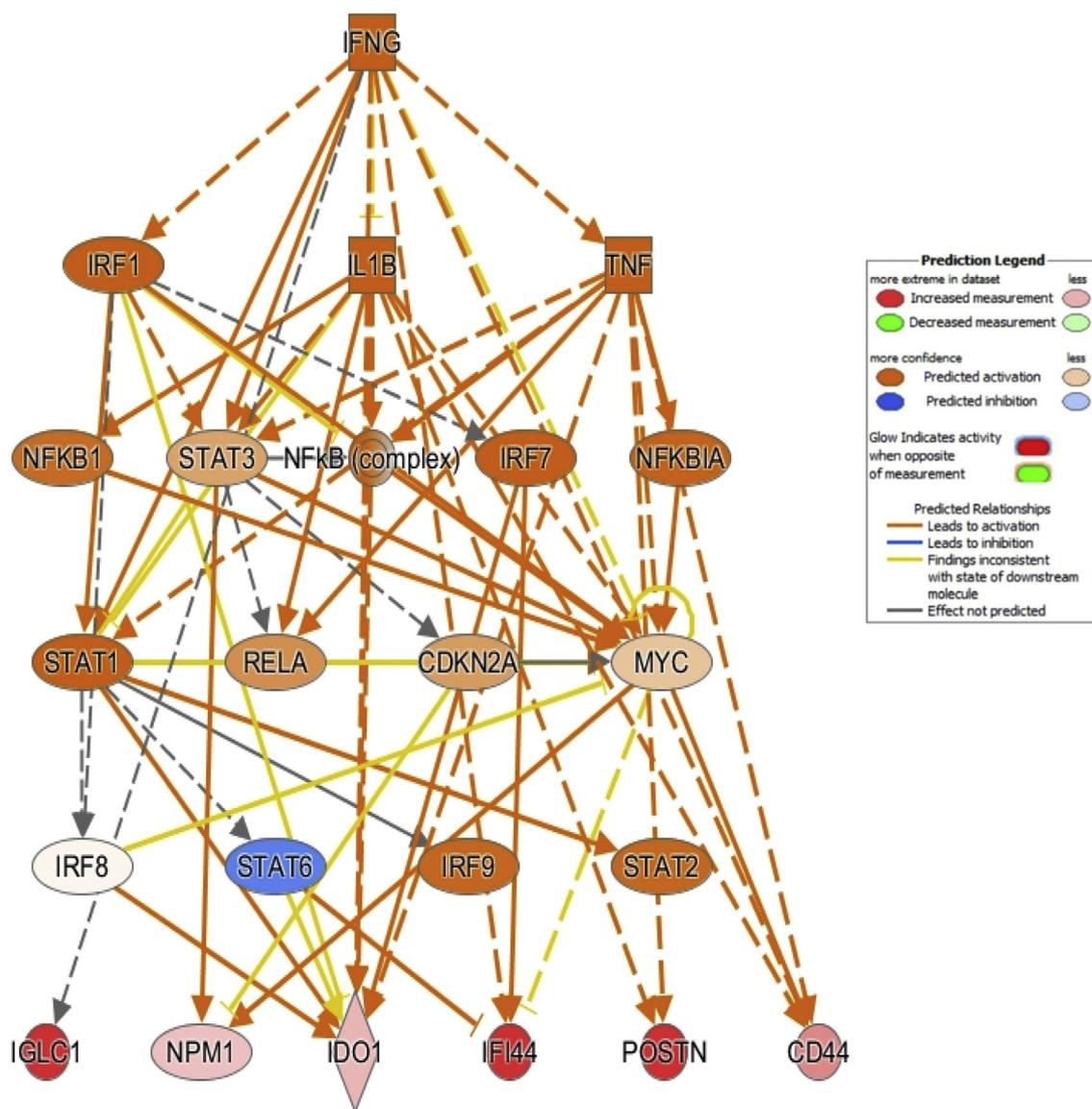
Figure 5. Ingenuity pathway analysis of several candidate genes with oncogenic and tumor suppressing properties downstream of IFNγ signaling. Prediction legend below shows relations of genes.

(Figure 7). Lastly, we studied TRIM gene expression in other disease samples to elucidate if the differential TRIM expression we observed is unique to DM. We conducted meta-analyses on synovial and blood samples from rheumatoid arthritis patients, bronchoalveolar lavage and blood samples from sarcoidosis patients, skin samples from systemic scleroderma patients, and blood samples from Kawasaki patients. From our comparative analysis, there was no overlap in TRIM gene expression patterns in rheumatoid arthritis, sarcoidosis, systemic scleroderma, and Kawasaki with our DM skin and muscle biopsy analyses. We only found one instance of overlap; upregulation of TRIM21 (p = 3.97E-8, expr log ratio 0.158) in sarcoidosis blood samples.

4. Discussion

Our analysis revealed significant upregulation of several TRIM family genes in both skin and muscle samples. These included TRIM5, a RING domain-E3 ubiquitin ligase involved in retroviral defense. It acts as a pattern recognition receptor for retroviral capsids and stimulates MAPK- and NFKB-dependent intracellular inflammatory signaling upon actvation [44]. Similarly, TRIM14 stabilizes cyclic GMP-AMP synthase and perpetuates a stronger IFN signaling response, leading to improved innate immunity [45]. TRIM21 is a well-described, common autoantibody target, and is involved in innate immunity and antiviral defense with a role in the development of Sjogren's syndome [46, 47, 48]. TRIM38 has also been shown to be required for antigenic RNA/DNA-associated immune response [49, 50]. Taken together, these results suggests that upregulation leads to a more robust, maladaptive innate immune response that contributes to DM pathogenesis.

Our analysis also revealed significant downregulation of certain TRIM family proteins in both skin and muscle samples. TRIM73, which has previously been associated with Williams-Beuren syndrome, is being linked to DM pathogenesis for the first time through our analysis [51]. TRIM22, shown to be involved in IFN-mediated anti-HIV responses, interrupts HCV replication suggesting strong anti-viral properties [20, 52].
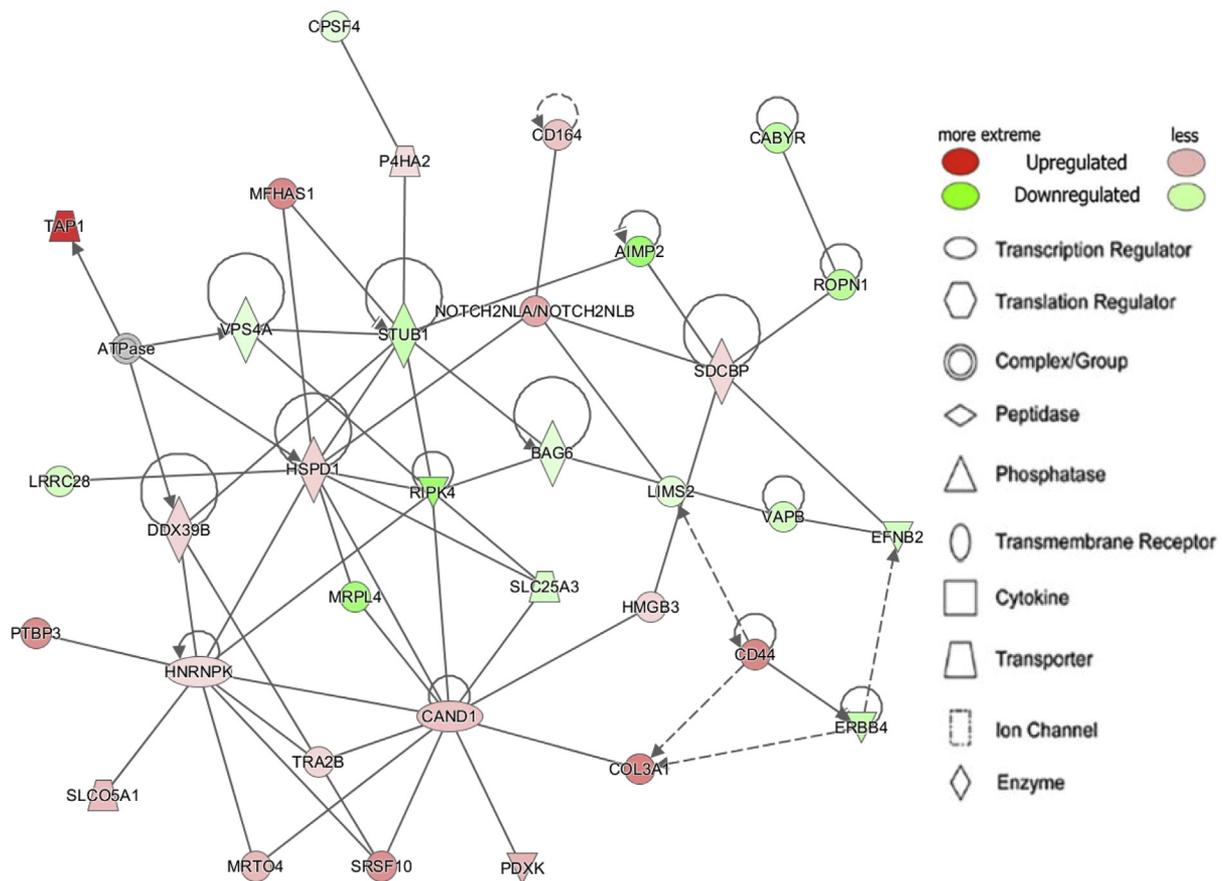
Figure 6. Cell death and survival, cell morphology, and RNA processing network identified in dermatomyositis muscle analysis. Legend illustrates class of the gene. Red indicates upregulation and green downregulation, with shade depicting magnitude of change. Solid and dashed lines depict direct and indirect, respectively, relationship between genes. Figure was generated using Ingenuity Pathway Analysis.

TRIM25 activates the Retinoic-Acid-Inducible Gene 1 (RIG-I) signaling pathway to induce antiviral activity, recently being shown to be inhibited in dengue virus infected cells, permitting uninterrupted viral activity [53, 54]. TRIM27 negatively regulates NOD2, whose dysfunction can lead to severe inflammatory disorders [55]. Interestingly, TRIM27 also positively regulates TNFα-induced apoptosis; deficiency of which is associated with impaired apoptosis despite DM's association with malignancy [56].

Although the pathogenesis of DM is incompletely understood, current evidence suggests a prominent role for type 1 interferon signaling [57]. Type 1 IFN signaling creates a maladaptive immune response and is upregulated in other autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome, and systemic sclerosis [8, 9]. Our analysis demonstrates significant interferon, as well as immunologic, metabolic dysfunction, and tumorigenesis signatures in both DM skin and muscle tissues.

DM skin sample analysis demonstrated also a clear interferon signature. Top upregulated genes IFNλ1, IFNα2, and IFNγ resulted in increased expression of Type 1 interferon signaling-associated genes such as RSAD2, MX2, BST2, OAS2, and OASL. Specifically, IFNα signaling led to activation of several proteins with known pathological implications (Figure 2). For example, upregulation of TAP2, a transporter protein involved in MHCI antigen processing and pro-inflammatory chemokines, such as CXCL10, indicates communication between and recruitment of innate and adaptive immune cells, especially T cells. Aminoacyl-tRNA synthetase, or WARS, is upregulated by IFN signaling, though its pathogenic role remains unclear [58]. KLRC1 indicates NK cell involvement, but is also upregulated in Th1 and Th2 activation pathways. Expression

of interferon-stimulated gene 15 (ISG15), and related pathway proteins such as HERC5, are uniquely elevated in DM compared to other muscle diseases [15]. ISG15 and IFI44, two molecules upregulated in our dataset, are also upregulated in T-cell non-Hodgkin disease, peripheral and cutaneous T-cell lymphomas. Recent evidence suggests IDO1, an immunomodulatory enzyme that metabolizes tryptophan, is activated during tumorigenesis and aids in immune response evasion [59]. We also found activation of the pBH3-like pro-apoptotic gene APOC6 [60]. Lastly, we noted potential metabolic dysregulation through decreased expression of ACO1, which transports iron into cells and assists with mitochondrial DNA maintenance [61].

DM muscle sample analysis demonstrated top canonical pathways included the antigen presentation pathway (Figure 3), and communication between innate and adaptive immune cells. Top upstream regulators include IFNα2, IFNλ1, IFNα, and IFNγ, with resulting increase in expression of CCL5, CXCL10, IGHG1, IGHG3, TLR3, and HLA-A, HLA-C, HLA-DRB1, and DDX58. Again, we see IFNα-mediated upregulation of genes with known pathological implications (Figure 4). For example, CXCL10 is involved in communication between innate and adaptive immune cells and was upregulated in both cutaneous and muscle samples. Immunoglobulin heavy constant gamma 3 (IGHG3) is similarly involved innate and adaptive immune cell communication and is also upregulated in both rheumatoid arthritis and systemic lupus erythematosus [62]. Increased expression of HLA -A, –C, and -DRB1 (an HLA type associated with autoimmunity) entails greater antigen presentation. Increased expression of C1QB, a key component of the complement system, has been noted in systemic autoimmune disorders [63]. DDX58, otherwise known as RIG-I, is a pattern recognition receptor whose
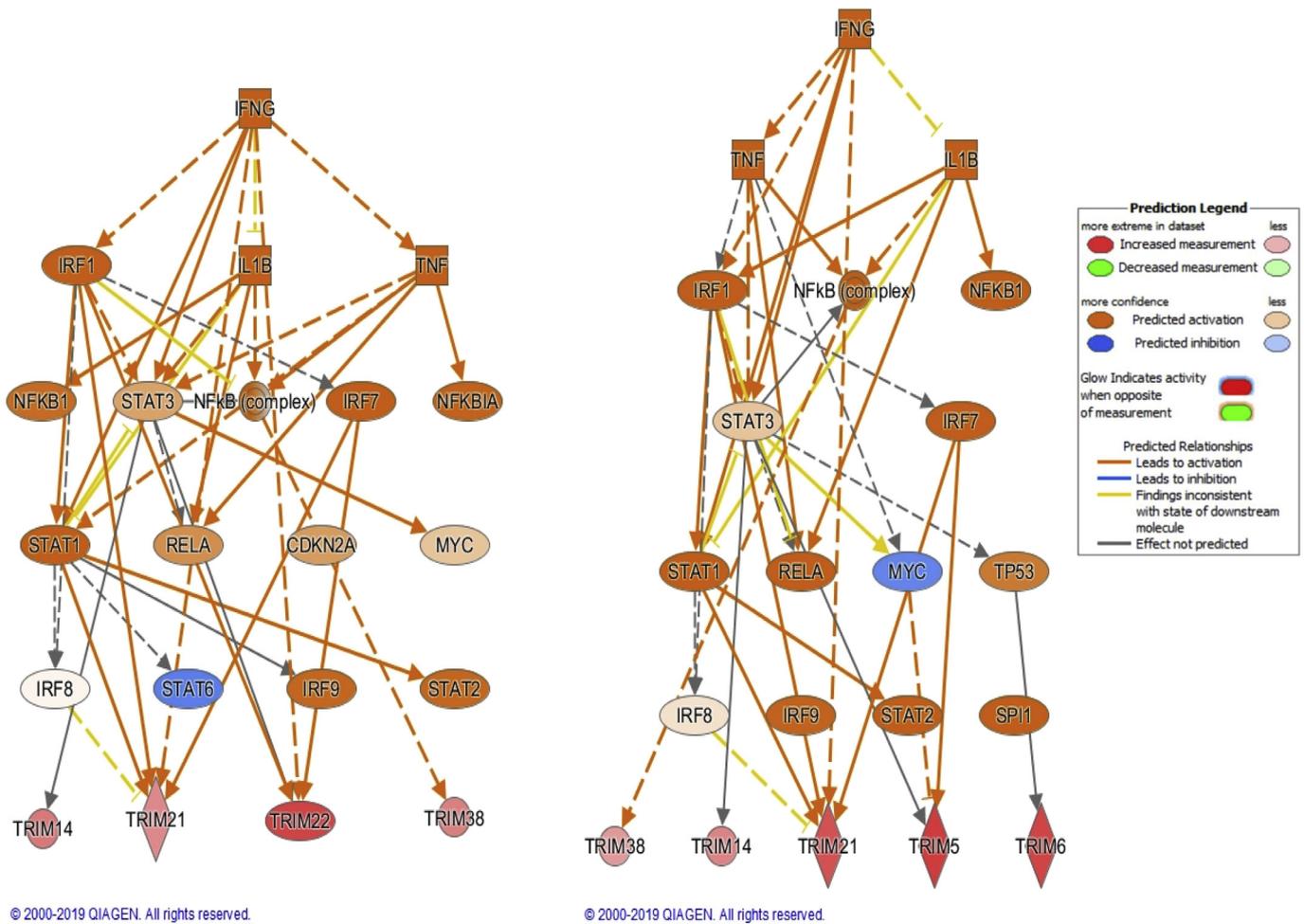
Figure 7. Interferon-γ induction of TRIM gene expression in skin (left) and muscle (right) samples from dermatomyositis patients. Relationship between genes shown by legend. Figure was generated using Ingenuity Pathway Analysis.

aberrant activation can cause autoimmunity [64]. RIG-I appears to play a significant role in DM but not in polymyositis or inclusion body myositis [16].

Interestingly, we note activation of various tumorigenesis pathways from our DM muscle biopsy analysis. Immunoglobulin lambda constant 1 (IGLC1) is part of the innate immune response but is also upregulated in chronic myeloid leukemia, T-cell lymphoma, and T-cell non-Hodgkin disease [65, 66]. IFI44, upregulated in both DM cutaneous and muscle samples, is also upregulated in T-cell non-Hodgkin disease, and peripheral and cutaneous T-cell lymphomas [67, 68]. Periostin (POSTN) is a secreted extracellular matrix protein part of the FAS1 domain, which functions in tissue development and regeneration, including wound healing. This protein binds to integrins to support adhesion and migration of epithelial cells and plays a role in cancer stem cell maintenance and metastasis [69, 70]. Single nucleotide polymorphisms of the POSTN gene have been associated with breast cancer susceptibility while being a negative prognostic factor in multiple epithelial ovarian cancers [69, 70].

We also found activation of MYC proto-oncogene expression, which is known to associate with increased malignancy risk in DM. Further tumorigenesis pathways identified in our dataset include CD44, NPM1, and IDO1. CD44 is a cell surface adhesion receptor typically overexpressed in different cancer cells and regulates proliferation, migration, and metastasis; conferring poor prognosis particularly for ovarian cancers [71, 72, 73]. Specifically there is an abundance of highly proliferative subpopulation of CD44+/CD177+ "cancer-initiating" cells, targeted

inhibition of CD44 has demonstrated success in treating ovarian cancer [73]. Overexpression of NPM1 is another marker of progression in solid tumors such as breast, hepatocellular, and ovarian carcinomas [74, 75]. Indoleamine 2,3-dioxygenase 1 (IDO1) is a tryptophan catabolic enzyme that is also implicated in tumorigenesis via promotion of immune tolerance to tumor antigens [76]. IDO is a target for anticancer therapy and inhibition results in rapid regression of tumors [76]. The presence of IDO1 in DM may represent a new therapeutic target for DM-associated tumors.

Interestingly, DM skeletal muscle analysis demonstrated dysregulation of genes related to cellular metabolism. We found that IFNα decreased expression of transferrin receptor (TFRC), ferroxidase (FXN), apolipoprotein C2 (APOC2), enolase 3, (ENO3), and fumarylacetoacetase (FAH) (Figure 4). TFRC and FXN are responsible for cellular and mitochondrial iron transport, respectively. APO2 activates lipoprotein lipase to hydrolyze triglycerides to fatty acids, as an essential energy source for skeletal muscles following mitochondrial oxidation. ENO3 is an enzyme expressed in skeletal muscle involved in development and regeneration [77]. FAH is involved in tyrosine catabolism and decreased activity result in the accumulation of succinylacetone, maleylacetoacetate, and fumarylacetoacetate, the latter presenting a mutagenic potential [78]. We also found IFNα-related upregulation of pannexin 1 (PANX1). Along with connexin, PANX1 is a major protein channel and is crucial for skeletal muscle organogenesis and maintenance. Under pathological conditions, altered PANX1 expression promotes muscle atrophy by stimulating inflammatory activity [79]. Last, we found reduced expression of

hexokinase, a key enzyme in glucose metabolism, and glycine hydroxy-ymethyltransferase. However, it is not known if downregulation of these enzymes is a cause or marker of DM muscle atrophy progression.

There are a few limitations with our study. As discussed above, DM is increasingly being defined by subtypes based on autoantibody types such as anti-Mi2, -MDA5, -NXP2, -TIF1, and -SAE antibodies [80]. The studies we found in GEO did not stratify samples based on subtype, so we analyzed aggregated results and our observations pertains to generalized mechanisms of DM pathogenesis. Additionally, as GEO did not state treatment status of DM patients, it is possible that some of the expression results were affected by the treatment regimen, especially immunosup-presives. Additionally, other comorbidities could also confound the effects of genetic changes outlined above. Moreover, the lack of serological details of the patients studied also is a limitation of the current study. Future studies should address these limitations by analyzing our top gene candidates across DM subtypes defined by myositis-specific autoanti-bodies and stratify patients based on treatment regimens.

5. Conclusion

Dermatomyositis (DM) is a chronic, autoimmune condition involving multiple organ systems and is associated with increased risk of malignancy. Our data demonstrate that type 1 interferon signaling may play a significant role in the autoimmune pathogenesis of DM, and identify several potential therapeutic targets including CXCL10, ISG15, IDO1 and DDX58. We saw upregulation of previously described oncogenes including IGLC1, POSTN, MYC, CD44, NPM1 and IDO1, and these genetic changes may, at least in part, explain the increased risk in malignancy seen in DM. We also show evidence of metabolic dysregulation and loss of skeletal muscle integrity in DM. Last, we identified TRIM family members expression as having differential tissue and disease-specific roles in DM pathogenesis, suggesting potential diagnostic and therapeutic implications.

Declarations

Author contribution statement

Jihad Aljabban: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Saad Syed, Wael Jarjour: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Sharjeel Syed, Michael Rohr, Noah Weisleder, Kevin E. McElhanon, Laith Hasan, Kalyn Hoffman, Maryam Panahiazar, Isaac Neuhaus, Susan Kim: Analyzed and interpreted the data; Wrote the paper.

Laraib Safeer: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Nabeal Aljabban, Mohamed Mukhtar, Nikhil Adapa, Zahir Allarakhia: Analyzed and interpreted the data.

Dexter Hadley: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Competing interest statement

The authors declare the following conflict of interests: Noah Weisleder; [founder of TRIM-edicine Inc].

Additional information

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