Oncogenomic analysis identifies novel biomarkers for tumor stage mycosis fungoides

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Abstract

Patients with mycosis fungoides (MF) developing tumors or extracutaneous lesions usually have a poor prognosis with no cure has so far been available. To identify potential novel biomarkers for MF at the tumor stage, a genomic mapping of 41 cutaneous lymphoma biopsies was used to explore for significant genes.

The gene expression profiling datasets of MF were obtained from Gene Expression Omnibus database (GEO). Gene modules were simulated using Weighted Gene Co-expression Network Analysis (WGCNA) and the top soft-connected genes (hub genes) were filtrated with a threshold (0.5). Subsequently, module eigengenes were calculated and significant biological pathways were enriched based on the KEGG database.

Four genetic modules were simulated with 3263 genes collected from the whole genomic profile based on cutoff values. Significant diseases genetic terminologies associated with tumor stage MF were found in black module. Subsequently, 13 hub genes including CFLAR, GCNT2, IFNG, IL17A, IL22, MIP, PLCG1, PTH, PTPN6, REG1A, SNAP25, SUPT7L, and TP63 were shown to be related to cutaneous T-cell lymphoma (CTCL) and adult T-cell lymphoma/leukemia (ATLL).

In summary, in addition to the reported genes (IL17F, PLCG1, IFNG, and PTH) in CTCL/ATLL, the other high instable genes may serve as novel biomarkers for the regulation of the biological processes and molecular mechanisms of CTLT (MF/SS).

Abbreviations: ATLL = adult T-cell lymphoma/leukemia, CTCL = cutaneous T-cell lymphoma, GEO = gene expression omnibus, IFN-γ = interferon gamma, iNOS = inducible nitric oxide synthase, KEGG = Kyoto Encyclopedia of Genes and Genomes, MF = mycosis fungoides, PLCG1 = phospholipase Cγ1, SEA = staphylococcal enterotoxin-A, SS = Sezary syndrome, WGCNA = Weighted Gene Co-expression Network Analysis.

Keywords: gene co-expression, Gene Expression Omnibus database, mycosis fungoides, tumor stage

1. Introduction

Mycosis fungoides (MF), also known as granuloma fungoides, is the most common form of cutaneous T-cell lymphoma (CTCL) and represents a complex series of diseases with various manifestations and treatment considerations.[1] MF has the long-term natural progression, a few years or sometimes decades of characteristics and the development of more infiltration plaques and eventually the formation of tumors, also known as tumor stage MF.[2] It is a relatively rare non-Hodgkin's lymphoma with a stable incidence of

0.36/10^5 from 1973 to 1992.[3] The rate of progression of the disease is variable, so patients may express patches, plaques, and tumors simultaneously in different areas of their skin and initially exhibit extracutaneous involvement.[4,5]

Previous studies of MF have shown that the most important prognostic indicators of survival are the type and extent of tumor involvement and the manifestation of extracutaneous disease.[6,7] However, due to the low resolution of comparative genomic hybridization techniques reported in previous studies, identification of specific genes involved in disease progression and prognosis remains very limited.[8,9] The development of whole genome analysis techniques has enabled the more complete and accurate characterization of human tumors with the goal of providing prognostic markers and targets for directed therapeutic intervention.[10]

In this study, a genetic array profile of numerous tumor-stage MF samples was evaluated in order to analyze genetic abnormalities in MF patients and to explore potential key genes. In addition, specific genetic changes were investigated that may help to understand disease progression and prognostic judgment.

2. Materials and methods

2.1. Datasets collection

The Gene Expression Omnibus (GEO) database is an international public repository that archives and freely distributes high-throughput gene expression and genomics datasets, designed to facilitate the sharing of genomic and clinical data between researchers. Microarray expression profile of tumor stage MF, obtained from GEO database with access number GSE18098,[11] was used to identify high- or low-expression genes based on the

average cutoff value of the copy numbers. This data profile was composite of 41 patients (22 males and 19 females) with an age range of 17 to 84 years. This study follows the dissemination and application policy requirements of GEO public data and has been approved by the Ethics Committee of the Institute of Biomedicine Research of the Chinese Academy of Medical Sciences and Peking Union Medical College. All genetic material used in the dataset was collected from the peripheral blood of patients with tumor stage MF. And genomic DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the protocol supplied by the manufacturer. The whole genome analysis of patient samples was processed via the Human Genomic CGH 44K microarray platform (Agilent Technologies, Palo Alto, CA).

2.2. Gene co-expression analysis and genome module detection

The gene co-expression network is a multidirectional relationship diagram, in which each node corresponds to a gene. If there is a significant relationship between 2 nodes, then the 2 nodes are connected to each other with an edge.[12] In contrast to standard analysis of differential gene expression, which attempts to detect the association of individual genes with the disease, this strategy aims to distinguish higher-order relationships among gene products.[13] The module eigengenes is defined as the important component of the corresponding module of the expression matrix.[14] An important goal of co-expression network analysis is to detect subsets of modules that are highly linked to each other, which is considered as a group of closely co-regulated genes that focus on the coherence of gene network modules.[15]

An important step in the detection of modular aggregates is the use of network proximity metrics to cluster genes into network modules. In short, if a pair of genes i and j are closely linked, they are functionally highly correlated. In general, the maximum expression similarity (s_{ij}) between 2 genes is 1 and the minimum similarity is 0. Typically, Weighted Gene Co-expression Network Analysis (WGCNA) uses the topological overlap matrix (TOM) [a_{ij}] as a measure of the proximity among genes[16] and can also be defined using a weighted network: $a_{ij=}(s_{ij})^{\beta}$, where the power β

is the soft thresholding parameter.[14] The method of dynamic branch cutting is used to define the branches of cluster tree as generated gene modules.[17] Next, the gene within the given module is summarized with the module eigengene, which can be considered as the best summary of the standard module expression data and is defined as the first major component of the normalized expression pattern.[18]

2.3. Metabolic function of gene modules

Enrichment analysis of each gene module is annotated, visualized, and further explored using a database, based on a background list of all the genes on the array. The exact Fisher's test results were adjusted with Benjamini-Hochberg method to measure the significant level of enrichment terminologies. After that, the core net-map of enrichment results was constructed to explore important metabolic function due to a single gene may interact with more than one terms.

2.4. Hub genes identification

Hub genes were defined as those genes that are highly connected to others within a genetic module. In order to elucidate the importance of highly connected genes and to identify their molecular functions, several screened hub genes of each genetic module were searched in PubMed database. And potential novel progression and prognostic biomarkers that haven't been reported in tumor stage MF would be found with this identify principles. Gene expression values and corresponding coefficient variations were calculated for each hub gene.

3. Results

3.1. Highly soft-connected genes

After processing of the expression profile dataset with the gene co-expression analysis procedure, a total of 3263 genes were collected with a cutoff value based on the R package WGCNA. Subsequently, each one of the genes was annotated and located in its own chromosome with a polar diagram (left part of Fig. 1).
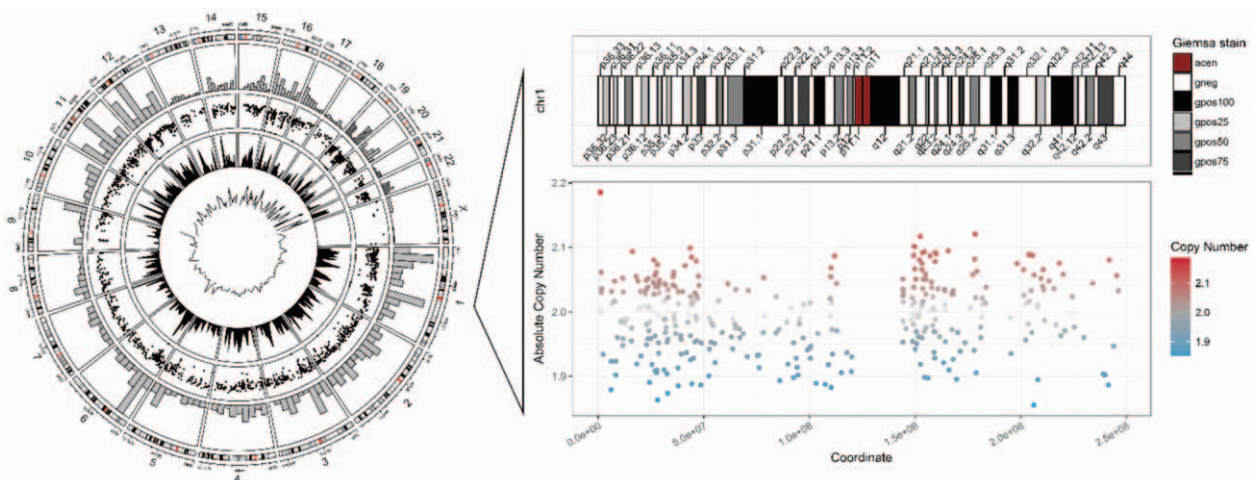
Figure 1. Location of collected genes on their own chromosome and the ideograph of chromosome 1. (Left part of Figure 1: polar ideograph includes the distribution characteristics of 3263 genes collected on 24 chromosomes. The first ring is the ideograph of chromosomes; the second ring represents the histogram of the gene counts; the third ring displays the location of genes corresponding to the distance from the neighboring regions (log-based); the forth ring calculates and adds the genomic density track of the genes; the inner density line represents the genomic instability. Right part of the Figure 1: ideograph and absolute copy number aberrations of chromosome 1 correspond to the P-value of each own genes.

Figure 2. Genes' co-expression network and global overview of the total and screened genes. A, Collected gene numbers for each chromosome. B, Co-expression netw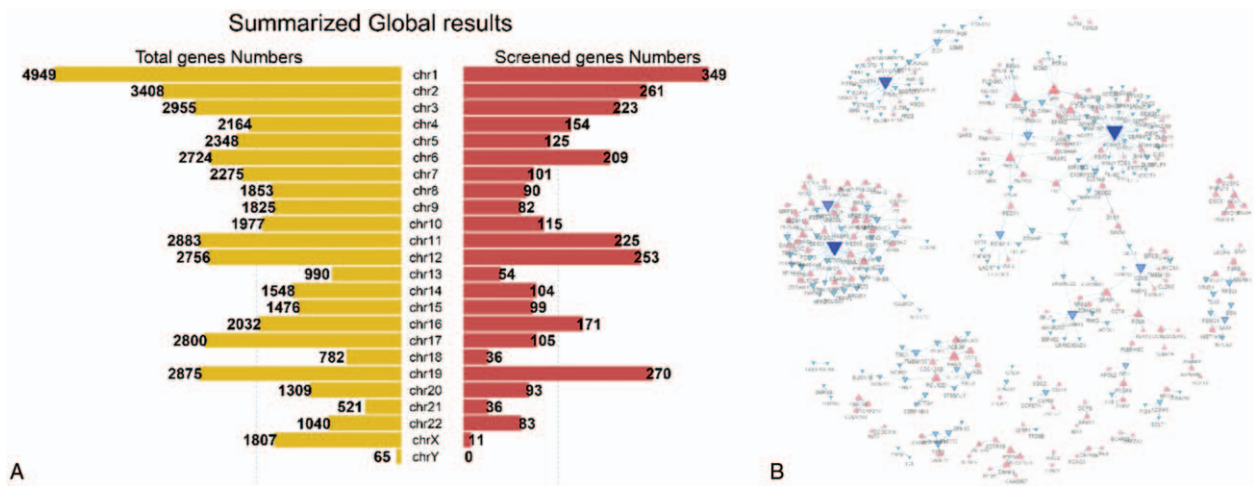ork for a total of 3263 genes. The red and blue triangles representing high and low copy number genes, respectively, and saturation of the color corresponding to the connected level.

These results showed that most of our screening genes are located on chromosomes 1, 2, 3, 6, 11, 12, and 19 (Fig. 2A). However, the most unstable chromosomes are 15, 16, 19, 20, and 22. The ideogram of chromosome 1 with cytogenetic band information was shown in the right part of Figure 1.

3.2. Co-expression modules related to tumor stage MF

To identify the functional modules of patients with tumor stage MF, co-expression analysis of the 3263 genes was performed in WGCNA (Fig. 2B). Forty-nine hub genes were found in this co-expression network including ACBD6, ADAMTS7, ANKRD35,
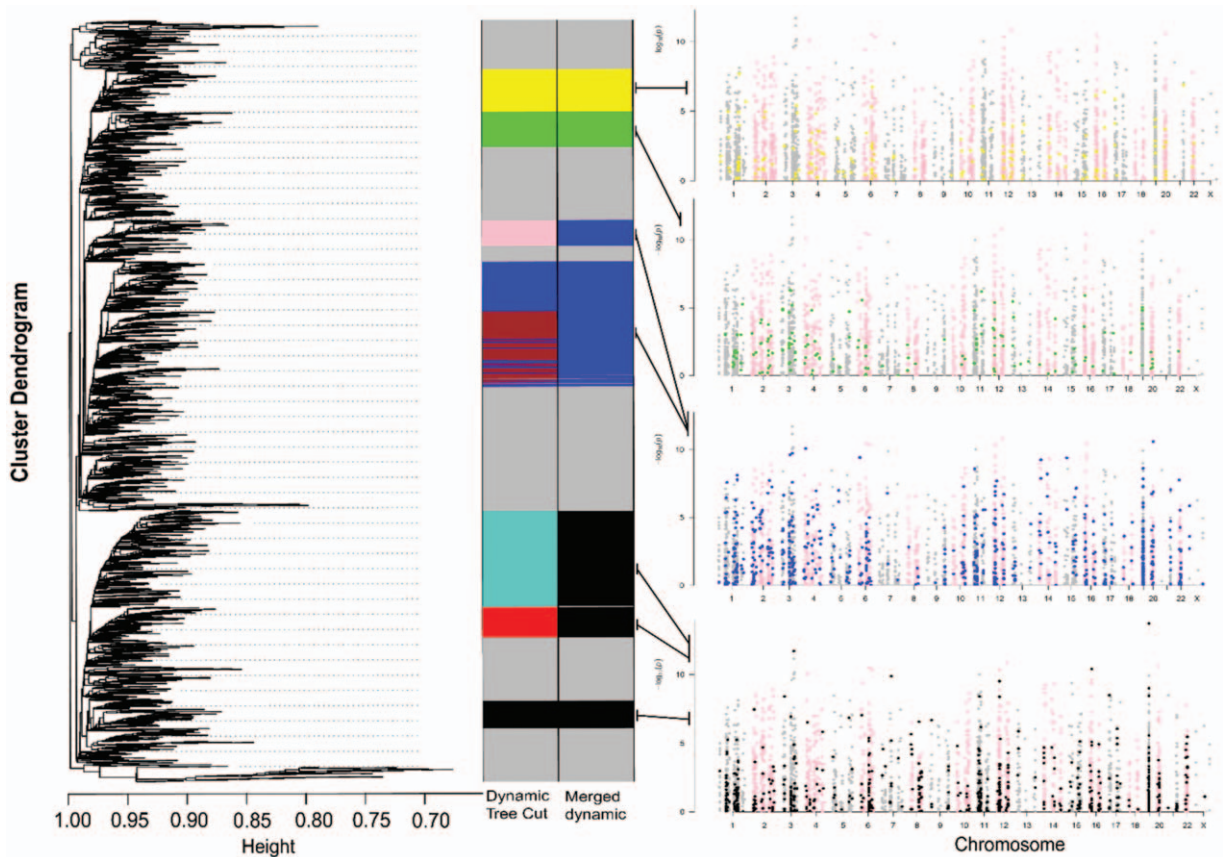
Figure 3. Dendrogram plot of modules detection and Manhattan plots. (Left part of Figure 3: Network analysis of gene expression in tumor stage MF identifies distinct modules of co-expression genes. Each leaf (short vertical lines) in the dendrogram corresponding to a gene and the branches are expression modules of highly interconnected groups of genes with a color to indicate its module assignment. Right part of Figure 3: Manhattan plot of each module including the distribution of its own assigned genes in the chromosomes. Each point in the module color representing a gene compared to the background genes.

BTBD9, CD8B, CEP68, CYP4F12, CYP4F3, DACT3, DNAH8, DPH1, EN1, ETV6, FIZ1, FXR2, GABRB1, GAS6, GPR84, HAO1, HFE2, INHBB, KCNJ14, METTL7B, MYO1F, NR1H3, PCK1, PCSK4, PCSK5, PLA2G4, PNPLA1, POLN, POLR2D, PRR12, PSORS1C1, RPS28, SLC10A1, SLC1A6, SLC23A3, SLC6A13, SOS1, SPOCK2, SPR, ST3GAL2, SUPT7L, THRB, TMEM156, TREML1, ZIC4, and ZNF232.

Modules generation for these collected genes were performed using the Scale-free Topology Criterion with a power of β=10 and merged with a threshold of 0.5. Finally, in addition to the grey module, which included genes that did not belong to any of the other modules, 4 genetic modules were identified with a threshold of cut-height = 0.98 and a minimum module size of 80 (left part of Fig. 3). In addition, we constructed Manhattan plots with information of chromosomes for each gene module, in which gene position and P-value (P-value was transformed to – log10(P) in y-axis of plots) in their annotation file, respectively (right part of Fig. 3).

3.3. Genome module functional analysis

In order to further explore the relationship between the gene modules and tumor stage MF, we conducted a network analysis of specific gene-network related diseases based on a significant level of $P < .05$ and key words such as lymphoma. The results showed high degree of correlation and significant level within themselves. Five major hub genes were listed along with notable pathways in each module (Table 1). Additionally, we found powerful evidence and specific gene-related disease associated with MF through disease-gene-network analysis in black module (Table 2). Such as adult T-cell lymphoma/leukemia (ATLL), Sezary syndrome (SS), and mycosis fungoides/Sezary syndrome nitric oxide synthase (NOS).

After the previous investigation of the gene modules, we performed an enrichment analysis of the black module using disease-gene-network and Kyoto Encyclopedia of Genes and Genomes (KEGG) database, respectively. The enrichment map was a sufficient way to identify the relationship between terminologies and hub genes. We found that important genes such as IL17A and IFNG were strongly associated with ATLL, CTCL and SS (Fig. 4A). Moreover, genes such as PTH, IL22, GCNT2, PTPN6, and immunologic deficiency syndromes were connected together. By the side, the enrichment pathway analysis

was performed to investigate significant signal transduction (Fig. 4B and Table 3). Notably, pathways of uptake and actions of bacterial toxins (hsa5339562) and neurotoxicity of clostridium toxins (hsa168799) were appeared in the results of KEGG pathway enrichment.

3.4. Potential novel genes involved in tumor stage MF

The major step of this study was to explore potential genes associated with tumor stage MF based on the results of enrichment analysis. With a cutoff value of 3, we found 13 prominent hub genes including CFLAR, GCNT2, IFNG, IL17A, IL22, MIP, PLCG1, PTH, PTPN6, REG1A, SNAP25, SUPT7L, and TP63. Among the gene list, PLCG1,[19] IL17F,[20] PTH,[21] and IFN-γ[22] had been reported to be functionally associated with CTLT and/or SS. However, according to our understanding, there was no direct evidence implicating the other genes are susceptible biomarkers for tumor stage MF.

4. Discussion

Our study analyzed a genome-wide analysis of chromosomal alterations in a group of 41 confirmed MF patients with tumor stage. A primary goal of this investigation was to mine most of the unstable gene copy numbers and to find potential novel biomarkers for the tumor stage MF. The diagnosis of MF presents some difficulties due to its highly variable clinical manifestation. At present, more than 50 clinical and pathological changes of MF have been reported in the published literatures.[23] Therefore, exploring potential novel biomarkers are valuable for diagnosis and treatment of tumor stage MF.

WGCNA is a commonly used data mining method, especially for studying the network relationship between genes and biological processes in genomic applications based on the analysis of the correlation between variables.[24] Using this technique, in the regions of the chromosomes that are not consistently expressed, we found several potential genes may be used as novel biomarkers in diagnosis and treatment of tumor stage MF. Phospholipase C, γ1, also known as PLCG1, can be activated by non-/receptor tyrosine kinases. Functional studies shown that PLCG1 increased the CTCL proliferation and survival mechanisms by inhibiting the NFAT pathway and lead to CTCL cell proliferation and cell viability.[19] In addition,

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Table 1

Modules specific to tumor stage MF disease stage.

Module	Correlation	P	Top 5 Hub genes	Notable Pathways ($P < .001$)
Blue	0.96	0	AKAP3, APOL2, AQP8, ARVCF, ATOH8	Diseases of signal transduction; class I MHC mediated antigen processing and presentation; signaling by WNT; antigen processing; ubiquitination and proteasome degradation; interleukin-20 family signaling
Black	0.73	5.80E-08	ADMT7, AVIL, CD8B, CEP68, CLPB	RHO GTPase effectors; translation; transcriptional regulation by RUNX1; mitotic prophase; SUMOylation
Green	0.77	3.00E-09	ACTR1B, BTBD9, DNAH8, SLC39A11, ZNF589	Intracellular signaling by second messengers; platelet activation, signaling and aggregation; class A/1 (rhodopsin-like receptors); PIP3 activates AKT signaling; PTEN Regulation
Yellow	−0.89	4.20E-15	NRG4, GRIN2B, FIG4, ACS1, FBXW7	Intracellular signaling by second messengers; PIP3activates AKT signaling; PTEN regulation; negative regulation of the PI3K/AKT network; PI5P, PP2A, and IER3 regulate PI3K/AKT signaling

Correlation of the first module eigengene with diseased state. This table characterizes the modules by Kyoto Encyclopedia of Genes and Genomes pathway enrichment, lists the genes involved in the categories, and by top 5 connected genes according to their K means, which connotes the genes correlation with the module eigengene.

Table 2

Diseases gene enrichment analysis results of black module.

ID	Description	P	Genes
C0013080	Down syndrome	.04324	SPR/GNLY/REST/CHAT/COL6A1/FABP7/MAL/IFNAR1/LTBR/NFATC2/DPYSL4/ENO2/STIP1/TCN1/PRDX3/SNAP25/ACTB/MX1/RBM4/IFNG/AZGP1/CLDN8
C0023493	ATCLL	.04003	GCNT2/TNFRSF11B/CFLAR/C3/PTH/PTPN6/SUPT7L/ENO2/REG1A/SNAP25/PLCG1/NRSN1/OAS3/RPSA/ATF7IP/LRP1/ASPA/IFNG/FOS/PSMD9/TXNIP/MIP
C0014072	Experimental autoimmune encephalomyelitis	.01142	TRPM4/SLC25A37/GCNT2/IL11RA/NTRK1/IL22/FABP7/CFLAR/IL17A/TP63/C3/NFATC2/IL25/CLDN11/REG1A/VPS52/RPSA/ZNF436/IFNG/C5AR1/AIF1
C0023492	Leukemia, T-cell	.00283	CDC20/FOXE3/ELF2/PTH/PTPN6/SUPT7L/RAC2/REG1A/SNAP25/SLC1A6/NRSN1/OAS3/RPSA/ATF7IP/LRP1/TCL6/GTPBP4/PROCR/IFNG/TXNIP/MIP
C0021051	Immunologic deficiency syndromes	.04917	ADAM28/SLC25A37/CEACAM5/LTBR/ZAP70/CD63/LIG1/C3/EXOSC8/ZNF395/PTH/APCS/MXD1/RAC2/COX8A/IRAK4/VAMP8/GIPC1/IFNG/EBI3
C0206180	Ki-1+ anaplastic large cell lymphoma	.00330	CALM3/GNLY/MYH9/NTRK1/IL22/CFLAR/IL17A/EPHA8/TP63/ZNF395/NFATC2/PTPN6/IRF6/SNAP25/ZNF436/IFNG/FOS
C0079773	Lymphoma, T-cell, cutaneous	.02983	TBX21/HDAC1/IL22/CFLAR/IL17A/TP63/PTPN6/SUPT7L/REG1A/PLCG1/PTK6/IFNG/FOS/HIST4H4
C0035372	Rett syndrome	.00219	HIST1H4E/NTNG2/RUNX2/PTH/ENO2/ZNF436/ACTB/HIST1H4C/HIST4H4/UBE2I/HIST1H4H
C0036920	Sezary syndrome	.02076	TBX21/GCNT2/NTRK1/IL22/IL17A/PTPN6/SUPT7L/PLCG1/KIR3DL2/IFNG
C0024291	Lymphohistiocytosis, hemophagocytic	.00088	LAMP1/GNLY/STXBP2/ZNF395/PTPN6/APCS/ASCL1/IFNG/MIP
C0012236	DiGeorge syndrome	.04164	TMEM132A/HIRA/SULT1E1/RAC2/ZNF74/DGCR6
C0549463	X-linked lymphoproliferative disorder	.01825	GCNT2/IL17A/PTPN6/APCS/IFNG
C0751674	Lymphangioleiomyomatosis	.01825	RPS6KB1/CALM3/GCNT2/TNFRSF11B/DES
C2316212	Cryopyrin-associated periodic syndromes	.01077	IL17A/PSTPIP1/IRAK4/IFNG
C0221269	Pseudolymphoma	.00206	SPHK2/RANGAP1/TP63/PTPN6
C0333997	Lymphoid hyperplasia	.00826	IFNG/TXNIP/MIP/ICOSLG
C1619738	Immune reconstitution inflammatory syndrome	.01266	IL22/IL17A/IFNG
C0265202	Seckel syndrome	.02044	MCPH1/DNMT1/GCNT2
C0862196	MF/SS NOS	.02350	IL17A/SUPT7L/PLCG1
C0085859	Polyglandular type-I autoimmune syndrome	.04228	PTH/CYP1A2/TMPRSS3
C0494261	Combined immunodeficiency	.04228	GCNT2/IL17A/IFNG
C1960272	Latent autoimmune diabetes mellitus in adult	.03738	GAD1/IFNG
C1262117	Fungal keratitis	.04475	IL17A/IFNG

ATLL = adult T-cell lymphoma/leukemia, MF = mycosis fungoides, NOS = nitric oxide synthase, SS = Sezary syndrome.
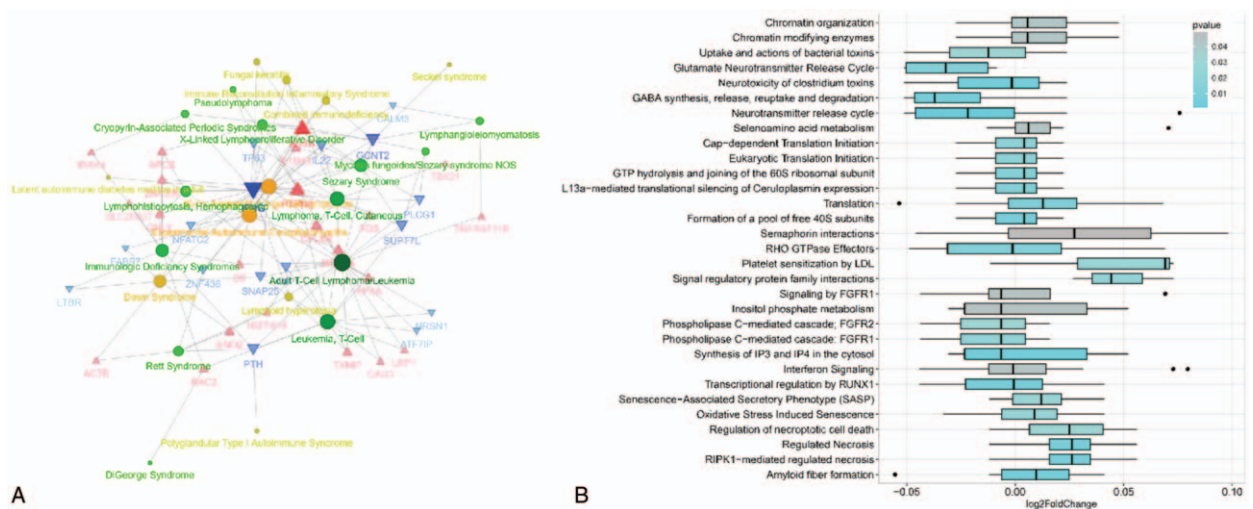
Figure 4. MF related functional terminology network map and significant pathways. A, Diseases gene enrichment analysis network. The triangles representing the genes in black enrichment module, red color representing high-expression genes, blue color representing low-expression genes. The size of a node is weighted by the power of the gene interacted with other genes or terminologies. B, The magnitude of gene expression change compared all background genes is represented by the horizontal box. And the significant levels represented by the legend's color saturation.

Table 3

KEGG pathways analysis results of black module.

ID	Description	P	Genes
195258	RHO GTPase effectors	.01323	FMNL3/CDCA8/CALM3/SPC25/RANGAP1/MYH9/CDC20/HIST1H4E/PPP2CB/TUBA4A/DYNC1LI2/ROPN1/RAC2/HIST2H2BE/NCF2/ACTB/HIST1H2AE/CENPT/HIST1H4C/HIST4H4/HIST1H4H
72766	Translation	.01065	EIF3F/PPA2/QARS/RPL13/GFM1/RPL32/MRPL23/RPL18/MRPS35/EIF3G/RPS3/MRPL15/LARS2/MRPS17/RPSA/VARS2/RPLP1/MRPS21/RPL11/EIF3K
8878171	Transcriptional regulation by RUNX1	.00592	SMARCC2/HDAC1/HIST1H4E/ELF2/PSMD11/RUNX2/NFATC2/HIST2H2BE/ARID2/HIST1H2AE/UBC/HIST1H4C/IFNG/HIST4H4/HIST1H4H/CLDN5/RING1/PSMD9
3247509	Chromatin modifying enzymes	.04176	SMARCC2/HIST1H2AM/REST/HDAC1/HIST1H4E/CDK4/SUPT7L/HIST2H2BE/ARID2/ATF7IP/SETDB1/ACTB/HIST1H2AE/HIST1H4C/HIST4H4/BRPF1/HIST1H4H
4839726	Chromatin organization	.04176	SMARCC2/HIST1H2AM/REST/HDAC1/HIST1H4E/CDK4/SUPT7L/HIST2H2BE/ARID2/ATF7IP/SETDB1/ACTB/HIST1H2AE/HIST1H4C/HIST4H4/BRPF1/HIST1H4H
913531	Interferon signaling	.04388	TRIM35/IFNAR1/NUP153/PTPN6/EIF4E2/IRF6/PLCG1/OAS3/IFITM1/MX1/UBC/IFNG/TRIM31
977225	Amyloid fiber formation	.00562	HIST1H4E/APCS/HIST2H2BE/HIST1H2AE/SEMG1/UBC/CST3/HIST1H4C/HIST4H4/HIST1H4H
2559580	Oxidative stress induced senescence	.02449	HIST1H4E/CDK4/HIST2H2BE/HIST1H2AE/UBC/HIST1H4C/FOS/HIST4H4/HIST1H4H/RING1
72689	Formation of a pool of free 40S subunits	.00690	EIF3F/RPL13/RPL32/RPL18/EIF3G/RPS3/RPSA/RPLP1/RPL11/EIF3K
156827	L13a-mediated translational silencing of ceruloplasmin expression	.01290	EIF3F/RPL13/RPL32/RPL18/EIF3G/RPS3/RPSA/RPLP1/RPL11/EIF3K
72706	GTP hydrolysis and joining of the 60S ribosomal subunit	.01367	EIF3F/RPL13/RPL32/RPL18/EIF3G/RPS3/RPSA/RPLP1/RPL11/EIF3K
72613	Eukaryotic translation initiation	.02001	EIF3F/RPL13/RPL32/RPL18/EIF3G/RPS3/RPSA/RPLP1/RPL11/EIF3K
72737	Cap-dependent translation initiation	.02001	EIF3F/RPL13/RPL32/RPL18/EIF3G/RPS3/RPSA/RPLP1/RPL11/EIF3K
2559582	SASP	.02814	HIST1H4E/CDK4/HIST2H2BE/HIST1H2AE/UBC/HIST1H4C/FOS/HIST4H4/HIST1H4H
2408522	Selenoamino acid metabolism	.04334	QARS/RPL13/RPL32/RPL18/RPS3/RPSA/RPLP1/RPL11/GNMT
112310	Neurotransmitter release cycle	.00074	GAD1/CHAT/ALDH5A1/GLS2/SLC6A13/SLC1A2/SNAP25/SLC1A6
373755	Semaphorin interactions	.04927	MYH9/PLXNB1/CD72/DPYSL4/RAC2/TYROBP
1855204	Synthesis of IP3 and IP4 in the cytosol	.00416	CALM3/INPPL1/ITPKA/PLCZ1/PLCG1
1483249	Inositol phosphate metabolism	.04474	CALM3/INPPL1/ITPKA/PLCZ1/PLCG1
5654736	Signaling by FGFR1	.04474	FGF3/PPP2CB/PLCG1/FGF6/UBC
5213460	RIPK1-mediated regulated necrosis	.00678	RIPK3/TRADD/CFLAR/UBC
5218859	Regulated necrosis	.00678	RIPK3/TRADD/CFLAR/UBC
888590	GABA synthesis, release, reuptake and degradation	.00559	GAD1/ALDH5A1/SLC6A13/SNAP25
210500	Glutamate neurotransmitter release cycle	.01315	GLS2/SLC1A2/SNAP25/SLC1A6
5339562	Uptake and actions of bacterial toxins	.03145	CALM3/VAMP1/SV2A/SNAP25
5675482	Regulation of necroptotic cell death	.03128	TRADD/CFLAR/UBC
5654219	Phospholipase C-mediated cascade: FGFR1	.02273	FGF3/PLCG1/FGF6
5654221	Phospholipase C-mediated cascade; FGFR2	.03128	FGF3/PLCG1/FGF6
391160	Signal regulatory protein family interactions	.02273	PTPN6/TYROBP/SIRPB1
432142	Platelet sensitization by LDL	.02682	PPP2CB/LRP8/PTPN6
168799	Neurotoxicity of clostridium toxins	.00580	VAMP1/SV2A/SNAP25

SASP = senescence-associated secretory phenotype.

PLCG1 had been reported play a key role in promoting cancer metastasis, and blocking its expression could prevent cancer from spreading. And the research is underway and this gene may lead to the development of new anti-cancer drugs.[25]

Interleukin 17A, the same as IL-17A or IL-17, is a pro-inflammatory cytokine produced by a group of T helper 17 cells in response to the stimulation of IL-23.[26] IL-17 function is also essential to a subset of CD4 + T-cells, which results in their roles has been associated with many immune/autoimmune related diseases including rheumatoid arthritis, asthma, lupus, psoriasis,[27] and multiple sclerosis.[28] And recent study have shown that staphylococcal enterotoxin-A (SEA) from the skin of infected CTCL patients and recombinant SEA stimulate the activation of STAT3 and the up-regulation of IL-17, indicating that SEA-producing bacteria promote carcinogenesis as well as activation of previously involved oncogenic pathways.[29]

In addition, Interferon gamma (IFN-γ), known as an immune interferon, has antiviral, immunoregulatory and anti-tumor

properties and is a product of lectin-stimulated human leukocytes and other antigen-stimulated lymphocytes.[30] It changes a variety of genes that produce different physiological and cellular responses. Such as increasing the antigen presentation and lysosomal activity of macrophages, activating inducible nitric oxide synthase (iNOS), and promoting the adhesion and binding required for leukocyte migration. Besides that, IFN-γ also stimulates macrophages via T-helper cells, making them more powerful in killing intracellular organisms promoting granuloma formation.[31]

However, there are some limitations in this study. First of all, we didn't explore the differences between tumor stage MF with other subtypes due to limited sample size and datasets. This has affected the potential implications of our findings in tumor stage MF. Second, the limitations of these genes involved in experimental measurements have prevented us from validating their comprehensive predictive ability, and further validation of the results has not yet been applied. The last potential limitation is that potential biomarkers obtained from peripheral blood may not necessarily translate into useful specific tumor tissue biomarkers.

Taken together, despite several identified biomarkers have been reported in our results, the other important genes (eg, CFLAR, GCNT2, IFNG, and so on) may also play important roles in biological processes and/or molecular functions. These findings may contribute to novel targets investigation for the diagnosis and therapy of tumor stage MF. In this study, we did not report additional comparisons because of the limited number of similar datasets and samples in published studies. Finally, further investigations and verification in the future needs to be compared with the reported biomarkers, such as PLCG1 and IFNG. Verifying the features of these genes reasonably represent the factors that influence the occurrence and development of the disease, so that an optimized risk-adapted diagnosis and treatment strategy can be designed.

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Writing – review & editing: Jianfang Sun.

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