Revista Argentina de Clínica Psicológica
2020, Vol. XXIX, N°3, 1091-1100
DOI: 10.24205/03276716.2020.938

# Identification of Key Regulators of Myeloproliferative Neoplasms Based on Relevant Network Analysis

**Zenghui Gu[a], Weiqun Mei[a], Zui Wang[a], Xinqiang Wang[a], Liangbang Wu[a], Zhaoming Ye[b*]**

**Abstract**

Myeloproliferative neoplasm is a common disease in the hematology department clinically with high mortality. In this study, we focused on the key regulators of myeloproliferative neoplasms to identify the molecular mechanisms of disease based on relevant network analysis. We performed differentially expressed gene analysis on disease samples of myeloproliferative neoplasms and clustered them into co-expression modules by WGCNA. A network analysis of functions and pathways was performed on the set of modules to identify the mechanism of action of key factors. Finally, based on the predictive analysis of multidimensional regulators, it was identified that a series of ncRNAs and transcription factors have potential regulatory effects on myeloproliferative tumor-associated factors. In result, we obtained 1069 differential genes, which were clustered into 9 modules and found 9 hub genes. Statistical analysis found that these modular genes participated in the negative and positive regulation of cytokine production and immune system process signaling pathways. In addition, network connectivity analysis screened 17 intracellular genes (including IL8, EP300 and CD74) regulating module genes. Finally, key regulators of significant regulatory block genes were determined, including 43 transcription factors (including E2F1, GATA1 and NFKB1) and 553 ncRNAs (including miR-139-5p, miR-106b-5p and miR-381-) 3p, etc.) These results can provide a new way for biologists and medical scientists to study myeloproliferative neoplasms as well as a valuable reference for subsequent treatment options.

**Keywords:** Myeloproliferative Neoplasms, Expression Disorder Factors, Key Regulatory Factors, Enrichment Analysis, WGCNA

## Introduction

Myeloproliferative neoplasms (MPNs) are a rare malignant tumor of the blood system characterized by bone marrow insufficiency, myelofibrosis, osteosclerosis, neovascularization, and spleen and extrahepatic extracorporeal hematopoiesis (Huberty et al.,2018; Selicean et al., 2018). The term myeloproliferative disease, born in 1951 and is renamed by the World Health Organization (WHO) as a myeloproliferative neoplasm (Thapa and Rogers, 2018; Barbui et al.,2016). Its four typical tumors, comprising chronic myeloid leukemia, polycythemia vera, essential thrombocythemia, and primary myelofibrosis, are all chronic hematological cancers with varying progression (Grinfeld et al.,2018). Among them, primary myelofibrosis (PMF) is the most invasive Philadelphia-negative myeloproliferative tumor with highest morbidity. Myeloproliferative neoplasms, a clonal disease derived from hematopoietic stem cells was characterized by proliferation of some or all of the myeloid lineages, and high probability of conversion turning into acute myeloid leukemia (O'Sullivan and Mead,2018; Li et al.,2018). According to the subtype of clonal disease, it is further classified into Philadelphia chromosome-positive chronic myeloid leukemia and chromosome-negative chronic myeloid leukemia (Swaminathan et al.,2018). However, the risk of leukemia transformation has been increasing with time and the incidence is extremely high (He et al., 2018). Thrombosis and autoimmune or inflammatory phenomena are common clinical symptoms of these clonal stem cell

*[a] The Third Department of Orthopedics, the 903th Hospital of the Logistic Support Force of the Chinese People's Liberation Army, 40, Airport Road, Xiacheng District, 310013, Hangzhou, Zhejiang, China.*
*[b] Department of Orthopaedics, The Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, 310009, China.88 Jiefang Road, Shangcheng District, Hangzhou City, Zhejiang, China*
**Corresponding Author: Zhaoming Ye*
*Email:yezhaoming@zju.edu.cn.*

diseases in addition, thrombosis is the most common complication in Philadelphia chromosome-negative myeloproliferative tumors. Recent studies have shown that changes in the expression pattern of CD markers imply abnormal frequency and function of blood cells, which leads to the complication of thrombosis (Shahrabi et al.,2018). Myeloproliferative neoplasms, also myeloid tumors, lack effective erythropoiesis and bone marrow coexistence of at least one hematopoietic lineage, with morphological features including hematopoietic dysplasia and other features characterized by marked proliferation (Kayano,2018). Several studies have shown that different manifestations of myeloproliferative neoplasms are associated with thrombotic propensity and risk of leukemia transformation, and activation of cytokine-independent signalling through the JAK/STAT cascade belongs to a prominent feature of the disease (Greenfield et al.,2018). Myeloproliferative neoplasms have an important association with eosinophilia, as evidenced by Afonso et al. (Strong et al., 2018). In addition, it has been found that myeloproliferative neoplasms probably develop into lymphoproliferative disorders. Patients carry the JAK2V617F mutation which is one of the most common genetic alterations in chronic lymphocytic leukemia (Mousinho et al., 2018). Allogeneic hematopoietic cell transplantation (HCT) is the only potential treatment for older patients with myeloproliferative neoplasms (Sengsayadeth et al.,2018).

Numerous biologists and medical scientists are looking for effective treatments for myeloproliferative neoplasms. According to studies by Medeiros LJ and other scholars, patients with myeloproliferative tumours respond well to tyrosine kinase inhibitor treatment, indicating that tyrosine kinase has a certain inhibitory effect on the development of myeloproliferative tumours (Xie et al.,2018). At the same time, yoga as a non-pharmaceutical strategy has been shown to improve the symptoms of other cancers and may be effective in improving the symptoms of patients with myeloproliferative tumours. Several scholars' studies showed that mutations in the ER chaperone calreticulin (CALR) are common in patients with this condition, which are capable of activating thrombopoietin receptors It is possibly a new potential treatment for myeloproliferative neoplasms (Pronier et al.,2018). Spanoudakis found that JAK2V617F is a gain in function point mutations in patients with myeloproliferative tumours, and that its hematopoietic function is disrupted at the cellular level, so osteoclast homeostasis caused by JAK2 can relieve the adjustment of hematopoietic stem cell niche in MPN patients (Spanoudakis et al.,2018). It is also shown that the β-3 sympathomimetic agonist can normalize blood cell counts in the blood of patients with myeloproliferative tumors to improve myelofibrosis, thereby inhibiting the disease (Drexler et al.,2018).

In light of relevant network analysis, we proposed a comprehensive method to explore the key regulatory factors of myeloproliferative tumors, which not only provides a new insight for the future treatment of it, but also brings abundant resources and guidance for further experiments to biologist.

## Result

### Gene differential expression analysis of myeloproliferative tumours

To explore the key virulence factors of myeloproliferative neoplasms, we selected 50 disease samples of the condition and 15 normal samples to construct gene expression profile with in-depth study, and gained 1069 differentially expressed genes whose characteristics were observed while these 1069 differential genes may be potential related genes of the disease. However, the analysis of gene differential expression requires further study.

### Co-expression behavior of related genes of myeloproliferative neoplasms

Through the differential gene expression of the disease, we have obtained the relevant pathogenic genes of myeloproliferative tumours, but the regulatory mechanisms and synergistic relationship between genes remain unclear. Therefore, we investigated the differential genes of myeloproliferative tumours, constructing the expression profiles of differential genes for co-expression analysis and then mined 10 modules whose genes all have a cooperative expression behavior (Figure. 1). At the same time, the 10 modules were screened, and the gray module was removed and finally 9 modules and 9 corresponding hub genes were obtained. In addition, the expression levels of key genes were verified by qPCR. We found that the expression trends of key genes are consistent with the results of the above analysis. These functional modular genes may be involved in different functions and pathways, probably representing diverse regulatory mechanisms mediating the development and progression of myeloproliferative neoplasms.

**The functions and pathways in which the modules are involved represent the pathogenesis of myeloproliferative neoplasms**

It is an important medium for identifying their pathogenesis to study the functions and pathways involved with genes. The results of enrichment analysis of module functions and pathways indicate that most of the functional modules are enriched for functions and pathways related to myeloproliferative neoplasms. We performed GO function and KEGG pathway enrichment analysis on the 10 functional modules obtained from the analysis, and obtained 24009 functions and 1119 KEGG pathway enrichment. These included 3,256 molecular functions (MF), 2,211 cell components (CC), and 18,542 biological processes (BP) involved in the gene (Figure. 2). Notably, all 10 modules are involved in the negative and positive regulation of cytokine production, which suggests that these two pathways may be the core signaling pathways for the development and progression of myeloproliferative neoplastic diseases. From the above data, we can find that these signaling pathways may be closely related to the occurrence and development of myeloproliferative tumors.

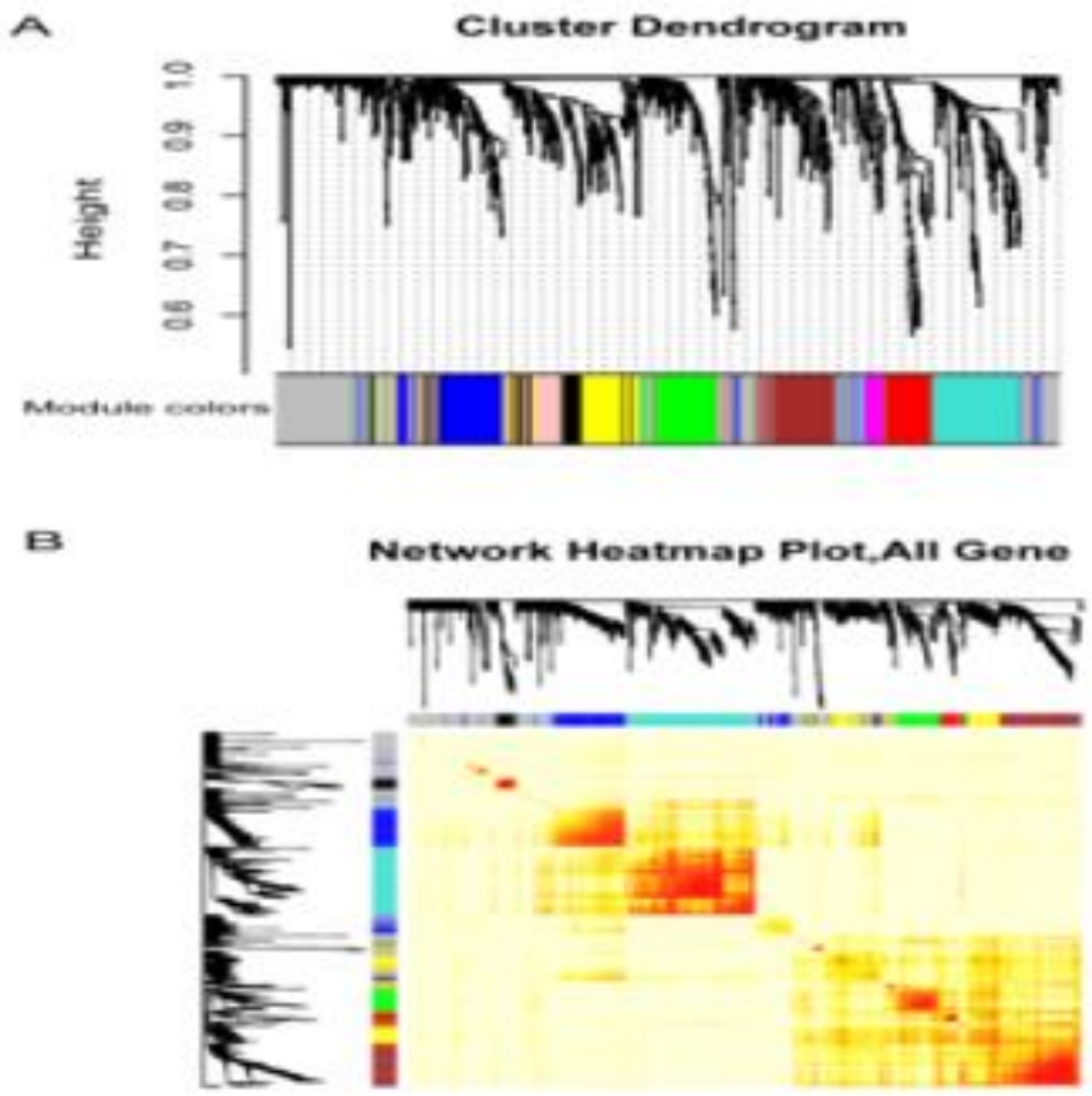


Figure 1. **Synergistic expression relationship clustering module of myeloproliferative tumors A. Clustering into 10 modules according to the differential gene co-expression relationship while one color represents a module. B. Heat map of the expression of the module gene in the sample. Genes related to myeloproliferative neoplasms visually exhibit a group expression in disease samples.**

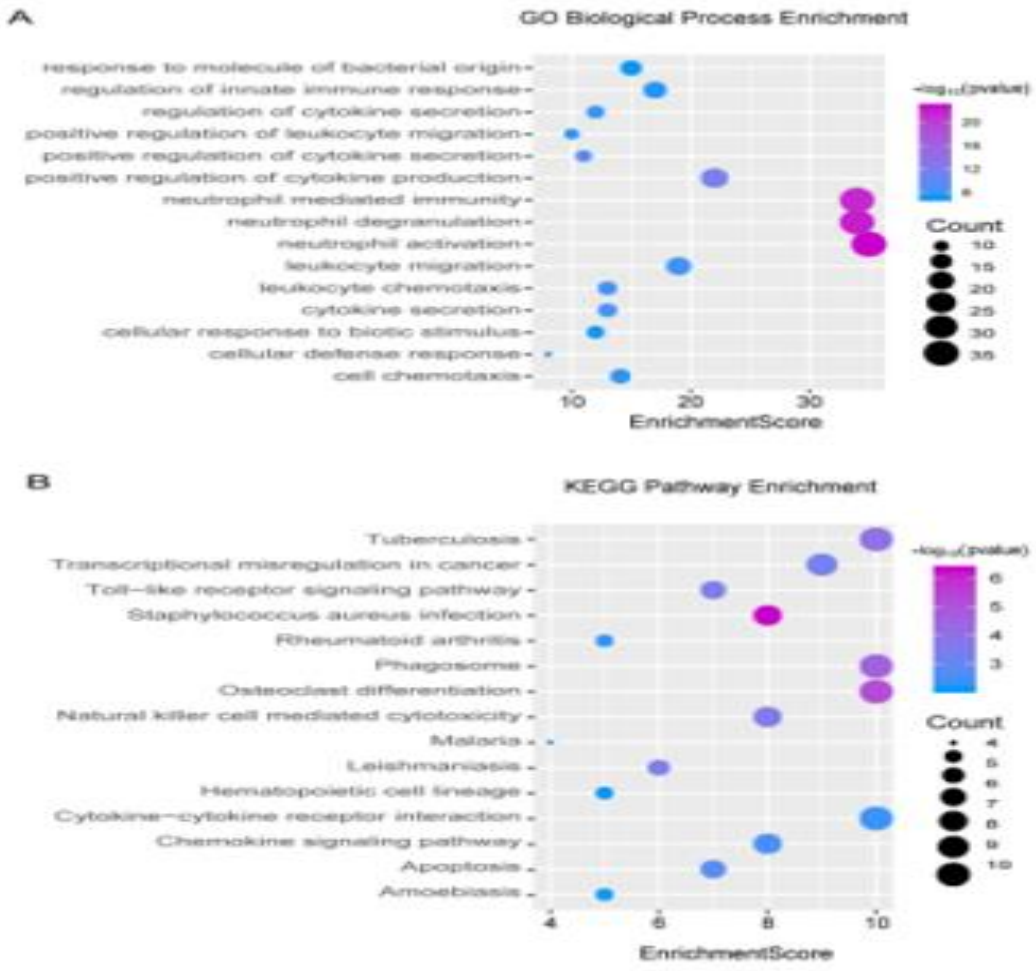


Figure 2. **Functional and pathways involved in modular gene identification of dysfunction modules for myeloproliferative neoplasms. A. Analysis excerpt of GO function enrichment in module genes the deeper the colors, the stronger the enrichment. The larger the circle, the greater the proportion of the module genes account for the entry gene of GO function. A. Analysis excerpt of KEGG pathway enrichment in module genes the deeper the colors, the stronger the enrichment. The larger the circle, the greater the proportion of the module genes account for the entry gene of KEGG pathway.**

**ncRNAs and TFs mediate myeloproliferative tumor dysfunction modules**

ncRNA has always been considered as an important regulator of the regulation of the occurrence and development of the disease. The scientific prediction of the ncRNA regulating the dysfunction module gene is helpful for us to further explore the pathogenic mechanism of myeloproliferative tumors. Therefore, we performed a pivot analysis based on the targeted relationship between ncRNA and genes. The predictions show that 553 pivot ncRNAs have significant regulatory effects on the module, with 706 Pivot-Module interactions in total (Figure 3). MiR-139-5p plays an important regulatory role in three dysfunction modules while miR-106b-5p affect two modules. Similarly, transcription factors are also important factors in the study of disease, and their dysregulation may lead to various diseases, including myeloproliferative tumors. Therefore, we performed a pivot analysis of the module on the basis of the regulatory relationship of the transcription factor to the gene. The results showed 43 pivotal transcription factors with significant transcriptional regulation of dysfunction modules of myeloproliferative neoplasms and 49 Pivot-Module interaction pairs (Figure 4). Statistical analysis of these transcription factor regulatory pairs revealed that E2F1 and GATA1 both significantly regulate the two dysfunction modules and are likely to be involved in the underlying pathogenesis of myeloproliferative tumors. The above data indicates that transcription factors may impact on the pathogenesis of the condition. These

data suggest that transcription factors probably play a big role in the mechanism of prostate cancer with significant regulatory effects on multiple dysfunction modules, identified as the core transcription factors. In addition, the network connectivity of the 11-dysfunction module was analyzed and 17 key endogenous genes were authenticated, including IL9, EP300 and CD74. These internal drive genes have high connectivity in the network module, and have significant regulatory effects on the immune mechanism of paroxysmal atrial fibrillation.

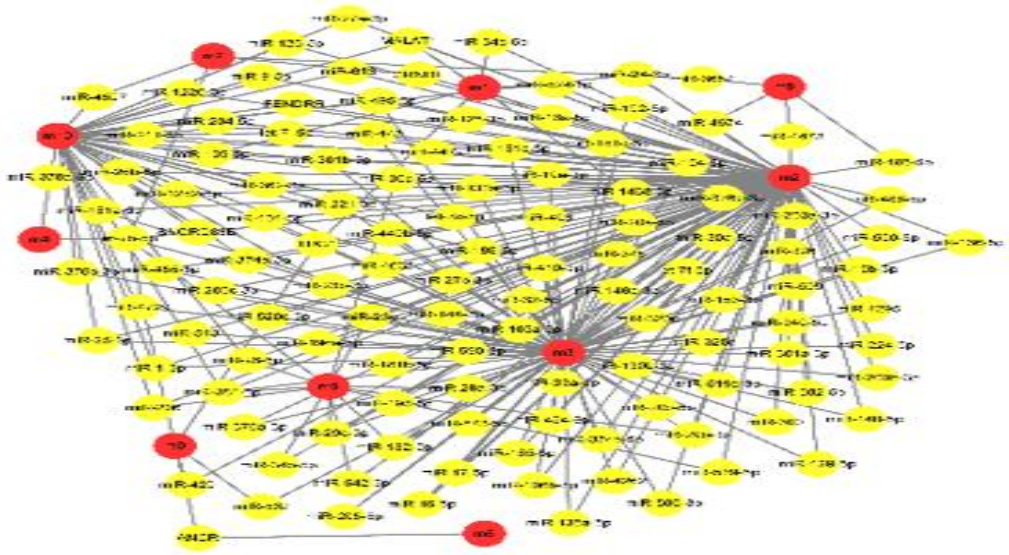

Figure 3. **ncRNA regulatory network map of myeloproliferative neoplasms with red circles representing modules and yellow circles representing corresponding ncRNAs.**

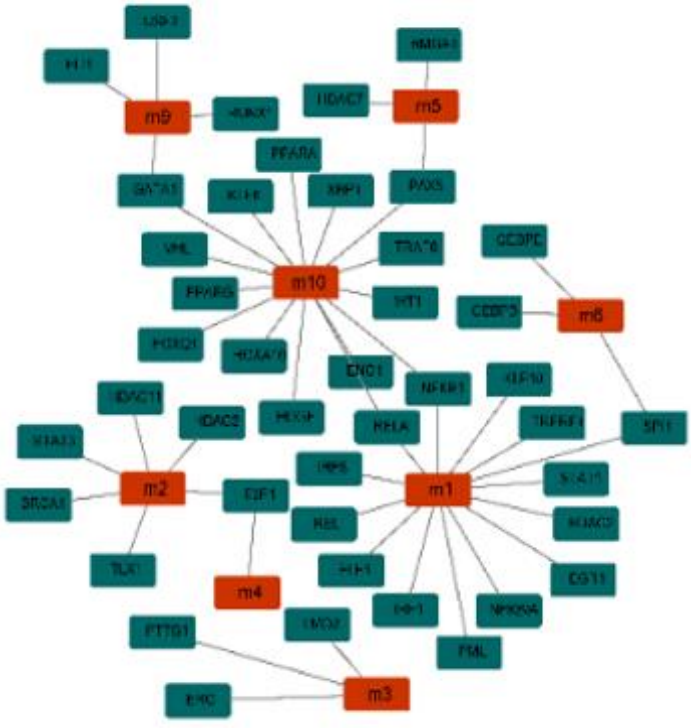


Figure 4. **Regulatory network diagram of transcription factor for dysfunction of myeloproliferative neoplasms, with orange rectangles symbolizing modules and blue rectangles standing for corresponding transcription factors.**

**Discussion**

Regarding myeloproliferative, a rare hematological malignant tumor, early risk factors are thromboembolic diseases, and most late risk factors are severe anemia and heart failure (Huberty et al.,2018). Although biomedical scientists have conducted in-depth researches on the pathogenesis and treatment mechanism of myeloproliferative neoplasms in recent years, there is still a lack of systematic and in-depth exploration of the key virulence factors of myeloproliferative neoplasms. In order to further understand the relevant pathogenesis of myeloproliferative neoplasms, we have integrated rich resources and conducted various analytical methods, such as gene differential analysis, and multi-dimensional integration of gene co-expression, transcriptional and post-transcriptional regulatory data.

For comprehensively exploring the mechanism of the potential pathogenic genes of myeloproliferative tumors, we analyzed the differential expression of disease gene expression profiles and obtained disease-related differentially expressed genes. The analysis found that the differentially expressed gene of JAK2 exists between the functional modules of myeloproliferative neoplasms, and gradually down-regulate grearly , which means that JAK2 gene has obvious impact on adjusting the pathogenesis of myeloproliferative tumors.. This conclusion was confirmed in studies by Swaminathan and Verstovsek et al. who emphasized that JAK2 mutations are the most common driving mutations in Ph-negative myeloproliferative tumors (Swaminathan et al.,2018). Besides, co-expression analysis of differentially related genes in myeloproliferative neoplasms was carried out. As a result, we obtained 10 co-expression modules whose genes had a synergistic expression. Besides, these 10 modules were screened, and the gray module was removed while eventually the 10 modules corresponding to 9 hub genes. Statistics found that the fourth module of the hub gene EBF1 is considered to have a certain promoting effect on the occurrence and development of myeloproliferative tumors. As confirmed in studies by scholars such as Duployez, the patients with the tumors was indentified secondary recessive genetic events in lymphoblastic transformation, including the transformation of EBF1(Duployez et al.,2016). In view of the enrichment analysis results, the genes in the 10 modules obtained from myeloproliferative tumors mainly participate in functional pathways such as negative and positive regulation of cytokine production and negative regulation of immune system process. In addition, we identified transcription factors that significantly regulate these 10 dysfunction modules, resulting in 43 transcription factors with 49 Pivot-Module interaction pairs, including E2F1 and GATA1 both regulating the two functional modules. The transcription factor E2F1 has a certain inhibitory effect in chronic myeloid leukemia (CML), which can act on CML treatment by tyrosine kinase inhibitors (Lee et al., 2016). GATA1 was found to decrease in expression in primary myelofibrosis, suggesting that it is also inextricably linked to myeloproliferative neoplasms (Yang et al.,2018). Therefore, these transcription factors are the core transcription factors that regulate the pathogenesis of the disease.

In addition, ncRNA has been recognized as an important regulator of the regulation of disease occurrence and development, and we carried out pivot analysis based on the targeting relationship between ncRNA and genes. The predicted results showed that 553 ncRNAs have significant regulatory effects on the module, involving 706 ncRNA-Module interaction pairs. Statistical analysis of the results revealed that miR-139-5p has a significant regulatory relationship with the three dysfunction modules so it is considered to be a key regulator of the core of myeloproliferative neoplasms. In a study of Kim VN et al, it was found that miR-139-5p negatively regulates the proliferation of hematopoietic stem and progenitor cells, and its down-regulation is associated with hematopoietic malignancies, so miR-139-5p can be identified as a key regulator of cell proliferationas in early hematopoietic process. (Choi et al.,2016). In addition, the miR-106b-5p also has a regulating effect on the two functional modules. It is evidenced that epigenetics is central to the progression of many cancers and miR-106b-5p positively promotes myeloproliferative neoplasms (Shen et al., 2017). Meanwhile, we also screened a series of genes with the greatest connectivity, which are core molecules of the regulation module, identified as endogenous genes. There were a total of 17 internal drive genes, which may be indicative of potential key regulatory moleculesm of the condition. Statistics on these internal drive genes have revealed that some of the internal drive genes can be identified with certain regulatory effect on the pathogenesis of myeloproliferative neoplasms. Among them, interleukin-8, a pro-inflammatory chemokine, activates multiple signaling pathways downstream of both receptors, and it is also the only cytokine associated with circulating cells (Corrado et al.,2014). According to the results of

Andersen CL et al., EP300 is identified as a common form of B cell non-Hodgkin's lymphoma common pathogenesis, and EP300 is also very likely to be involved in the pathogenesis of myeloproliferative tumors(Andersen et al., 2012).

The predicted series of regulatory factors in this study regulate the underlying pathogenesis of myeloproliferative tumors in a way. However, except for the above-mentioned key factors, other unmentioned ncRNAs and transcription factors may contribute to pathogenesis of myeloproliferative neoplasms, which requires further exploration. Conclusion: In general, our research based on the results of relevant network analysis to explore the potential key regulatory factors in its pathogenesis, which not only leaves a new idea for biologists and medical scientists to study the relevant pathogenesis of myeloproliferative tumors but also brings valuable reference for its subsequent treatment options.

## Materials and Methods

### Data resource

We collected expression microarray datasets from the NCBI Gene Expression Omnibus (GEO) database (Barrett et al.,2013) for samples of myeloproliferative neoplastic disease (including 50 disease samples and 15 normal samples), numbered GSE103176 (Zini et al.,2017). It contains the GPL data about mRNA and the GSE series matrix file (GPL13667) and then potential gene expression profile data for myeloproliferative tumors.

### Differential expression analysis

We performed a differential analysis to the normal and disease samples of the fiber layers in the mRNA, using the R language limma package (Ritchie et al., 2015). and then used the backgroundCorrect function to perform background correction and normalization of the data. The control probe and the low-expressed probe are filtered based on the normalize normalization method of the normalizeBetweenArrays function to obtain high quality standardized data. Finally, the differential expression mRNA of the data set was identified by the lmFit and eBayes functions of the R language limma package, respectively, and the differentially expressed genes with the parameter p value > 0.05 were obtained. In addition, we used the same method described above to perform differential analysis on miRNA data to obtain disease-related differentially expressed miRNAs.

### Co-expression analysis identifying relevant functional modules

First, the differential gene of myeloproliferative neoplasms was integrated, and the gene expression profiling matrix of 1069 differential genes was analyzed with WGCNA (Langfelder et al.,2008) to find a gene module for synergistic expression. The correlation coefficient weighting value is used, that is, the gene correlation coefficient is taken to the Nth power, and the correlation coefficient (Person Coefficient) between any two genes is calculated. The connections between genes in the network were subject to scale-free networks, making the algorithm more biologically meaningful. Then, a hierarchical clustering tree was constructed by correlation coefficients between genes. Different branches of the clustering tree represent different gene modules, and different colors represent different modules. 10 co-expression modules extracted were identified as significant dysfunctional modules of myeloproliferative tumor-associated genes.

### Functional and pathway enrichment analysis identifing dysfunction modules

Exploring the function and signal pathway of genes is often an effective means to study the molecular mechanism of diseases while those of module gene can characterize the dysfunction mechanism of module in the process of disease occurrence. Therefore, we performed enrichment analysis with respect to the Go function (pvalueCutoff = 0.01, qvalueCutoff = 0.01) and the KEGG pathway (pvalueCutoff = 0.05, qvalueCutoff = 0.2) for the 10 modular genes of myeloproliferative tumor with the R language Clusterprofiler package. According to the function and pathway involved in the module gene, it is identified as a related dysfunction module for myeloproliferative tumors.

### Identification of effect of the transcription factors and ncRNA on modules

All human transcription factor target data was downloaded and used in the general database TRRUST v2 database (Han et al.,2018) of transcription studies, and 43 transcription factors and 49 interaction pairs came out. Human ncRNA-mRNA data (score > 0.5) was then downloaded from the RAID 2.0 database (Yi et al.,2017), resulting in 706 interacting pairs involving 553 ncRNAs. Pivot analysis based on these interaction data was performed to identify the regulatory effects of these transcription factors and ncRNA on the modules. Pivot analysis refers to searching for

at least two interacting drivers with the module in the target pair and calculating the significance of the interaction between the driver and the module according to the hypergeometric test and screening TF and ncRNA with P value < 0.01 as the pivots of the significant regulatory module. Finally, via statistical analysis on pivots, ones that have a regulatory effect on more dysfunctional modules are identified as core pivots. In addition, Cytoscape (Carlin et al.,2017) is used for display and network analysis (including connectivity calculation), screening the genes with the greatest connectivity which is considered as the core molecule to regulate the progress of the module and identified as the intrinsic gene. screening the genes with the greatest connectivity which is considered as the core molecule to regulate the progress of the module and identified as the intrinsic gene. The identified 17 internal genes may represent potential key regulators of myeloproliferative tumors.

**qPCR assay detecting gene expression levels**

All blood samples were taken from Qinghai Infectious Disease Hospital, confirmed by experienced pathologists. All patients received informed consent. Human tissue samples were taken in accordance with international ethical guidelines for biomedical research involving humans and subjects. The study was approved by the Ethics Committee of Qinghai Infectious Disease Hospital, in accordance with the provisions of the Ethics Committee.

Specifically, total RNA in the blood is extracted, transcribed into cDNA with a reverse transcription kit and then subjected to a qPCR reaction using a SYBR-qPCR detection kit. The qPCR program begins denaturation step at 95 °C to activate the hot-start iTaqTM DNA polymerase in the initial 3 minutes, perform 45 denaturation cycles at 95 °C for 10 seconds, anneal and extend at 60 °C for 45 seconds. The internal reference gene is β-actin.

**Acknowledgement**

Zhejiang Provincial Committee of Medicine, Health and Planning, Project Name: LINC00858, feasibility report on the role and mechanism of ceRNA in regulating CDK14 expression in osteosarcoma, Project No. :2019329727

**References**

[1] Andersen CL, Hasselbalch H, Gronbaek K.2012; Lack of somatic mutations in the catalytic domains of CREBBP and EP300 genes implies a role for histone deacetylase inhibition in myeloproliferative neoplasms. *Leukemia research*. 36(4):485-7. DOI 10.1016/j.leukres.2011.11.018

[2] Barbui T, Thiele J, Gisslinger H, Finazzi G, Vannucchi AM, Tefferi A.2016 The 2016 revision of WHO classification of myeloproliferative neoplasms: Clinical and molecular advances. *Blood reviews.* ;30(6): 453-9.DOI 10.1016/j.blre.2016.06.001

[3] Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, et al.2013; NCBI GEO: archive for functional genomics data sets--update. *Nucleic acids research.* 41(Database issue): D991-5.DOI 10.1093/nar/gks1193

[4] Carlin DE, Demchak B, Pratt D, Sage E, Ideker T.2017; Network propagation in the cytoscape cyberinfrastructure. *PLoS computational biology*. 13(10): e1005598.DOI 10.1371/journal.pcbi.1005598

[5] Choi J, Kim YK, Park K, Nah J, Yoon SS, Kim DW, et al. 2016; MicroRNA-139-5p regulates proliferation of hematopoietic progenitors and is repressed during BCR-ABL-mediated leukemogenesis. *Blood*.128(17): 2117-29.DOI 10.1182/blood-2016-02-702464

[6] Corrado C, Raimondo S, Saieva L, Flugy AM, De Leo G, Alessandro R.2014; Exosome-mediated crosstalk between chronic myelogenous leukemia cells and human bone marrow stromal cells triggers an interleukin 8-dependent survival of leukemia cells. *Cancer letters*. 348(1-2):71-6. https://doi.org/10.1016/j.canlet.2014.03.009

[7] Drexler B, Passweg JR, Tzankov A, Bigler M, Theocharides APA, Cantoni N, et al. 2018. The sympathomimetic agonist mirabegron did not lower JAK2-V617F allele burden, but restored nestin-positive cells and reduced reticulin fibrosis in patients with myeloproliferative neoplasms: results of phase 2 study SAKK 33/14. *Haematologica.*

[8] Duployez N, Nibourel O, Ducourneau B, Grardel N, Boyer T, Bories C, et al.2016; Acquisition of genomic events leading to lymphoblastic transformation in a rare case of myeloproliferative neoplasm with BCR-JAK2 fusion transcript. *European journal of haematology.* 97(4): 399-402.DOI 10.1111/ejh.12752

[9] Greenfield G, McPherson S, Mills K, McMullin MF. 2018; The ruxolitinib effect: understanding how molecular pathogenesis and epigenetic

dysregulation impact therapeutic efficacy in myeloproliferative neoplasms. *Journal of translational medicine.* 16(1): 360.DOI 10.1186/s12967-018-1729-7

[10] Grinfeld J, Nangalia J, Baxter EJ, Wedge DC, Angelopoulos N, Cantrill R, et al.2018; Classification and Personalized Prognosis in Myeloproliferative Neoplasms. *The New England journal of medicine*. 379(15): 1416-30.DOI 10.1056/NEJMoa1716614

[11] Han H, Cho JW, Lee S, Yun A, Kim H, Bae D, et al.2018; TRRUST v2: an expanded reference database of human and mouse transcriptional regulatory interactions. *Nucleic acids research.* 46(D1): D380-D6.DOI 10.1093/nar/gkx1013

[12] He Z, Wang B, Chen L, Huang Y, Wang H, Yang M, et al.2018; MLL-PTD in a 13-year-old patient with blast phase myeloproliferative neoplasm: A case report. *Medicine*. 97(46): e13220.DOI 10.1097/MD.0000000000013220

[13] Huberty J, Eckert R, Larkey L, Gowin K, Mitchell J, Mesa R.2018; Perceptions of Myeloproliferative Neoplasm Patients Participating in an Online Yoga Intervention: A Qualitative Study. *Integrative cancer therapies*. 17(4): 1150-62.DOI 10.1177/1534735418808595

[14] Kayano H. 2018; [Myelodysplastic/myeloproliferative neoplasm: a histopathological review]. [Rinsho ketsueki] *The Japanese journal of clinical hematology*. 59(10): 2084-8.DOI: 10.11406/rinketsu.59.2084

[15] Langfelder P, Horvath S. 2008; WGCNA: An R package for weighted correlation network analysis. *BMC bioinformatics.* 9: 559.DOI 10.1186/1471-2105-9-559

[16] Lee SH, Um SJ, Kim EJ.2016; CBX8 antagonizes the effect of Sirtinol on premature senescence through the AKT-RB-E2F1 pathway in K562 leukemia cells. *Biochemical and biophysical research communications.* 469(4): 884-90.DOI 10.1016/j.bbrc.2015.12.070

[17] Li B, Mascarenhas JO, Rampal RK. 2018Leukemic Transformation of Myeloproliferative Neoplasms: Therapeutic and Genomic Considerations. *Current hematologic malignancy reports*. ;13(6): 588-95.DOI 10.1007/s11899-018-0491-5

[18] Mousinho F, Santos PSE, Azevedo AP, Pereira JM, Lemos R, Matos S, et al.2018; Concomitant myeloproliferative and lymphoproliferative neoplasms, distinct progenitors: A case report and review of the literature. *Molecular and clinical oncology*. 9(3): 347-9.DOI 10.3892/mco.2018.1682

[19] O'Sullivan J, Mead AJ. 2018.Heterogeneity in myeloproliferative neoplasms: Causes and consequences. *Advances in biological regulation.DOI 10.1016/j.jbior.2018.11.007*

[20] Pronier E, Cifani P, Merlinsky TR, Berman KB, Somasundara AVH, Rampal RK, et al.2018; Targeting the CALR interactome in myeloproliferative neoplasms. *JCI insight.* 3(22). DOI 10.1172/jci.insight.122703

[21] Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al.2015 limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic acids research*;43(7): e47.DOI 10.1093/nar/gkv007

[22] Selicean SE, Tomuleasa C, Grewal R, Almeida-Porada G, Berindan-Neagoe I.2018 Mesenchymal stem cells in myeloproliferative disorders - focus on primary myelofibrosis. *Leukemia & lymphoma.*: 1-10.DOI 10.1080/10428194.2018.1516881

[23] Sengsayadeth S, Gatwood KS, Boumendil A, Labopin M, Finke J, Ganser A, et al. 2018; Conditioning intensity in secondary AML with prior myelodysplastic syndrome/myeloproliferative disorders: an EBMT ALWP study. *Blood advances*. 2(16): 2127-35.DOI 10.1182/bloodadvances.2018019976

[24] Shahrabi S, Ehsanpour A, Heidary S, Shahjahani M, Behzad MM.2018; Expression of CD markers in JAK2(V617F) positive myeloproliferative neoplasms: Prognostic significance. *Oncology reviews*. 12(2):373.

[25] Shen N, Jiang L, Li Q, Cui J, Zhou S, Cheng F, et al. 2017; The epigenetic effect of microRNA in BCR-ABL1positive microvesicles during the transformation of normal hematopoietic transplants. *Oncology reports.*38(5):3278-84. https://doi.org/10.3892/or.2017.5966

[26] Spanoudakis E, Papoutselis M, Bazdiara I, Lamprianidi E, Kordella X, Tilkeridis C, et al.2018; The JAK2V617F Point Mutation Increases the Osteoclast Forming Ability of Monocytes in Patients with Chronic Myeloproliferative Neoplasms and Makes their Osteoclasts more Susceptible to JAK2 Inhibition. *Mediterranean journal of hematology and infectious diseases.* 10(1): e2018058.

*[27]* Strong C, Abecasis J, Afonso C, Trabulo M, Gouveia R. 2018; Endomyocardial Fibrosis with End-Stage Heart Failure as a Consequence of a Myeloproliferative Neoplasm with Hypereosinophilia. *The Canadian journal of*

*cardiology.* 34(9):1233 e13- e15.DOI 10.1016/j.cjca.2018.05.024

[28]Swaminathan M, Patel KP, Huynh-Lu J, Tang G, Zuo Z, Miranda R, et al.2018; Unique Case of Myeloproliferative Neoplasm with Two Rare Clonal Abnormalities: Rare JAK2 Exon 12 Mutation and Rare e14a3 (b3a3) BCR/ABL Fusion Transcript. *Acta haematologica*. 141(1): 23-7.DOI 10.1159/000494427

[29]Thapa B, Rogers HJ. 2018Cancer, Myeloproliferative Neoplasms. StatPearls. *Treasure Island* (FL).

[30]Xie W, Wang SA, Hu S, Xu J, Medeiros LJ, Tang G.2018; Myeloproliferative neoplasm with ABL1/ETV6 rearrangement mimics chronic myeloid leukemia and responds to tyrosine kinase inhibitors. *Cancer genetics*. 228-229: 41-6.DOI 10.1016/j.cancergen.2018.08.002

[31]Yang N, Park S, Cho MS, Lee M, Hong KS, Mun YC, et al.2018; GATA1 Expression in BCR/ABL1-negative Myeloproliferative Neoplasms. *Annals of laboratory medicine*. 38(4): 296-305.DOI 10.3343/alm.2018.38.4.296

[32]Yi Y, Zhao Y, Li C, Zhang L, Huang H, Li Y, et al.2017; RAID v2.0: an updated resource of RNA-associated interactions across organisms. *Nucleic acids research.* 45(D1): D115-D8.DOI 10.1093/nar/gkw1052

[33]Zini R, Guglielmelli P, Pietra D, Rumi E, Rossi C, Rontauroli S, et al. 2017; CALR mutational status identifies different disease subtypes of essential thrombocythemia showing distinct expression profiles. *Blood cancer journal*.7(12): 638.DOI 10.1038/s41408-017-0010-2