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Comprehensive Analysis of Multi-Regulator-Driven Dysfunctional Modules in Calculous Cholecystitis

Cuiping Sha[a#], Yanfang Qiu[b#], Shan Zhao[c*]

Abstract

Cholecystitis is inflammation of the gallbladder caused by obstruction of the cystic duct and bacterial invasion, and is often associated with gallstones, which is also called calculous cholecystitis. Its pathogenesis is regulated by genes and the environment. However, this has not been clearly stated in previous studies. Therefore, based on dysfunctional modules in cholecystolithiasis with cholecystitis, this study attempted to describe the multidimensional adjustment landscape of the disease from a global perspective. Functional modules were identified from tissue-specific expression proteins-associated interaction network in calculous cholecystitis. These tissue-specific expression proteins and their interactors often played an important role in calculous cholecystitis. Functional enrichment results indicated that they were mainly involved in various immune responses, inflammatory responses, cholecystokinin and cholesterol-related regulatory processes, which may represent a potential pathogenesis of calculous cholecystitis. Then, we identified transcription factors (TFs) (including E2F1, MYC and NFKB1, etc.) and ncRNAs (including CRNDE, miR-590 and miR-340, etc.) that have potentially important regulatory effects on calculous cholecystitis. These pivot regulators may manipulate genes in modules to mediate the occurrence of calculous cholecystitis. In addition, we used drug-target information to predict potential drugs for calculous cholecystitis, including Copper, Amitriptyline, Nortriptyline, and so on. These drugs may have certain pharmacological or toxicological effects on calculous cholecystitis, which requires further experimental exploration. Overall, based on a comprehensive functional module's analysis, we identified tissue-specific expression proteins, interactors and pivot regulators to analyze the underlying pathogenesis of calculous cholecystitis. The regulation of potential drugs also provided a valuable reference for drug developers to conduct drug relocation studies.

Keywords: Gallbladder calculus, Cholecystitis, Regulators, Dysfunctional modules, Potential drugs.

Introduction

Gallstone disease (GSD) is one of the most common biliary tract diseases worldwide, with an incidence of 10-15%, and is more common in women (Brazzelli et al, 2014).It is a complex disease that is affected by environmental and genetic factors. Symptoms include hypersaturation of cholesterol in bile, precipitation of excess cholesterol, nucleation caused by abnormal mucin, insufficient gallbladder movement and dysfunction of bile by kinetic protein factors and so on (Chuang et al, 2012; Chuang and Hsi,2013). According to the composition of the stones, gallstones can be divided into cholesterol gallstones (representing the main entity) and bilirubin (a pigment) stone, which may be its potential pathology (Lammert et al, 2016). After the stone is formed in the gallbladder, it can stimulate the gallbladder mucosa, which may not only cause chronic inflammation of the gallbladder, but also cause secondary infection after the stone is invaded in the neck of the gallbladder or the cystic duct,

[a]Department of Surgical, South District of Guang'anmen Hospital, China Academy of Traditional Chinese Medicine, Beijing,102600, China.
[b]Department of Kidney, South District of Guang'anmen Hospital, China A cademy of Traditional Chinese Medicine, Beijing,102600, China.
**Corresponding Author: Shan Zhao*
[#] The two authors contributes equally to this article.
Email:bjzs99@sina.com

leading to inflammation of the gallbladder. Epidemiological statistics show that up to 95% of cholecystitis is associated with complications of gallstones (Gotzky et al, 2013). Gallstones have become a risk factor for most cholecystitis and may gradually deteriorate into gallbladder cancer under this chronic stimulation. In patients with calculous cholecystitis, symptoms such as right upper quadrant pain, fever and leukocytosis often appear (Katabathina et al, 2015). Studies have shown that these symptoms of calculous cholecystitis are associated with many related genes, metabolic pathways and signaling pathways, involving in bile salt and cholesterol synthesis, lipid transport and various immune inflammatory responses (Hernandez-Nazara et al, 2006). The work of Balboa E et al. showed that the expression of the related gene Niemann-Pick C2 protein (NPC2) in the liver is an important factor in regulating dietary cholesterol metabolism and disposal as well as diet-induced cholesterol gallstone formation in mice (Balboa et al, 2012).

In addition, the ABCG5/G8-independent pathway plays an important role in regulating bile cholesterol secretion and transporting HDL-derived cholesterol into plasma to bile, which may be a potential mechanism for the formation of cholesterol gallstones in mice (Wang et al, 2016). Inhibition of SCF / c-kit signaling pathway can promote the reduction of interstitial cells of Cajal (ICCs, pacemakers and mediators of neuromuscular transmission of gastrointestinal motility) in gallstone disease, leading to gallbladder dyskinesia and gallbladder stones Occurs (Tan et al, 2014). On the other hand, in these gallstone patients, peroxisome proliferator-activated receptors (PPARs) regulate inflammation and lipid metabolism in cells via ATP-binding transporter A1 (ABCA1)-mediated pathways, which may be stones the underlying mechanism of gallbladder inflammation in patient (Lee et al, 2008). The evidence also shows that inflammation has a positive correlation and positive feedback relationship with the expression and activity of ABCA1[12]. In addition to ABCA1, ABCG5 and ABCG8 also play an important role in the pathogenesis of inflammatory diseases in patients with cholesterol-related stones, which is also potentially related to lipopolysaccharide (LPS (Yoon i et al, 2013). Lipopolysaccharide (LPS)-pathway proteins may also mediate the development and progression of gallbladder carcinoma (GBC) through these inflammation-related pathways (Van et al, 2016). In addition, increased E-cadherin and β-catenin and abnormal activation of the Sonic hedgehog signaling pathway and the ERK/MAPK pathway have also been shown to play an important role in the progression of cholecystitis in gallstones to gallbladder cancer (Puhalla et al, 2005; Xie et al, 2014; Buchegger et al, 2017)

Here, we proposed a comprehensive strategy based on dysfunctional modules to explore the underlying pathogenesis of calculous cholecystitis. Based on tissue-specific expression proteins in the gallbladder of three patients with calculous cholecystitis, we constructed a protein-protein interaction (PPIs) network and extracted functional modules in the network. Next, based on functional and pathway enrichment analysis, we examined and identified 24 dysfunctional modules. In our findings, related genes may be involved in these dysfunction modules and played an important role in mediating related functions and pathways, which in turn affect calculous cholecystitis. In addition, based on transcriptional and post-transcriptional regulation, we identified pivot regulators significantly regulated dysfunction modules, including transcription factors (TFs) and ncRNAs. They played a key role in the underlying pathogenesis of calculous cholecystitis. Finally, based on drug-target information, we predicted potential drugs that have regulatory effects on the module. These drugs may have pharmacological or toxicological effects on calculous cholecystitis and may serve as a reference for further research. In summary, our comprehensive strategy based on functional modules not only help to explore the underlying molecular mechanisms of calculous cholecystitis, but also provided abundant resources and guidance for biologists to further design experiments.

Materials and Methods

Data resources

The clinical diagnosis, pathological images and gallbladder tissue samples of 3 patients with calculous cholecystitis were collected. High-performance liquid Chromatography (HPLC) (Blum et al, 2014) and mass spectrometry were used to identify tissue-specific expression proteins in three disease samples. All experiments in this study were approved by the Ethics Committee and conducted in accordance with the guidelines of the National Health and Medical Research Council of China.

STRING database (Szklarczyk et al, 2015) is a search tool specifically designed to retrieve protein-protein interactions (PPIs). It provides the most comprehensive view of the currently available PPIs and is widely used for PPI analysis. In this study, PPIs with scores > 900 in human were obtained from the STRING database, a total of 10,514 proteins and 405,916 interaction pairs. The transcriptional and post-transcriptional regulatory relationships in

human were exacted from the TRRUST v2 database (Han et al, 2018) and the RAID v2.0 database (Yi et al, 2017), including 2,492 transcription factors (TFs) and 9,396 TF-target relationships, 5,431 ncRNAs and 431,937 ncRNA-mRNA/protein pairs (score > 0.5). In addition, all drugs and their target information data were downloaded from the Drugbank database (Law et al, 2014) for predicting potential drug and drug targets.

Identification of functional modules

In order to further explore the underlying pathogenesis of calculous cholecystitis, we first constructed a PPI network in calculous cholecystitis, which is composed of these gallbladder tissue-specific expression proteins and their reliable interactors identified from the STRING PPI network. Then, visualized with Cytoscape(Shannon et al, 2003) and mining the functional modules using ClusterONE(Nepusz et al, 2012). Finally, 24 modules with a number of genes > 50 were identified for further analysis.

GO and KEGG pathway enrichment analysis

Gene ontology (GO) (pvalueCutoff = 0.01, qvalueCutoff = 0.01) and KEGG pathway (pvalueCutoff = 0.05, qvalueCutoff = 0.2) enrichment analysis of genes were performed with the R language Clusterprofiler package (Yu et al, 2012). In addition, the functional enrichment analysis of network was performed by BinGO (Maere et al, 2005), a plug-in in Cytoscape.

Prediction of regulators and potential drugs

Regulators that significantly regulates the calculus dysfunction module were defined as pivot regulators. According to requirements, the number of interactions between each regulator and each module is greater than 2, and the significance of the interaction between the regulator and the module is calculated according to the hypergeometric test, p value < 0.01. Similarly, potential drugs for calculous cholecystitis were predicted based on drug target information.

Results

Identification of Gallbladder tissue-specific expression proteins

Observing the clinical diagnosis results of 3 patients (Table 1), multiple indicators have exceeded the standard to varying degrees. For example, the TG (triglyceride) values of these patients were slightly exceeded, the CHOL (total cholesterol) value of No.75508 was slightly exceeded. The pathological diagnosis results showed that the three patients (No. 75076, No.75508 and No.75133) were diagnosed with gallstones with chronic cholecystitis, and No.75508, No.75133 with mild and severe fatty liver, respectively.

To further explain the clinical results, we collected gallbladder tissue samples from these patients for proteolysis, high performance liquid chromatography and protein profiling (Supplementary Figure S1). It was found that there were 326 tissue-specific expression proteins in No.75076, 940 proteins in No. 75508 and 1030 proteins in No.75133 (Figure 2). As shown, these proteins have a high degree of overlap. In particular, there were 10 common tissue-specific expression proteins in three patients, suggesting that the potential pathogenic mechanisms of the three patients were highly similar. Finally, a total of 2014 tissue-specific expression proteins were obtained.

Table 1. Clinical diagnosis results of 3 patients

	No.75076	No.75133	No.75508
Disease	Gallbladder stones with chronic cholecystitis	Gallbladder Stone with chronic cholecystitis	Gallbladder stones with chronic cholecystitis
PMH	Grade-1 hypertensives	-	-
Age	50	29	64
Gender	male	female	female
Height (m)	1.6	1.62	1.6
Weight (kg)	68	50	75
B-Ultrasound	Gallstone	Gallstone	Gallstone
Pathological	Chronic cholecystitis	Chronic cholecystitis	Chronic cholecystitis
TG (0-1.7)	1.7	2.2	1.81
CHOL (2.59-6.47)	5.46	3.87	6.65
HDL-C (>1.04)	1.06	0.93	0.5
LDL-C (0-3.37)	3.74	2.31	4.8
Lipoprotein (0-300)	319.44	79.37	200
TBil (3.4-17.1)	17.8	19	10

TBA (0-9.67)	1.33	11.91	5.1
Blood sugar	4.26	5.08	4.5

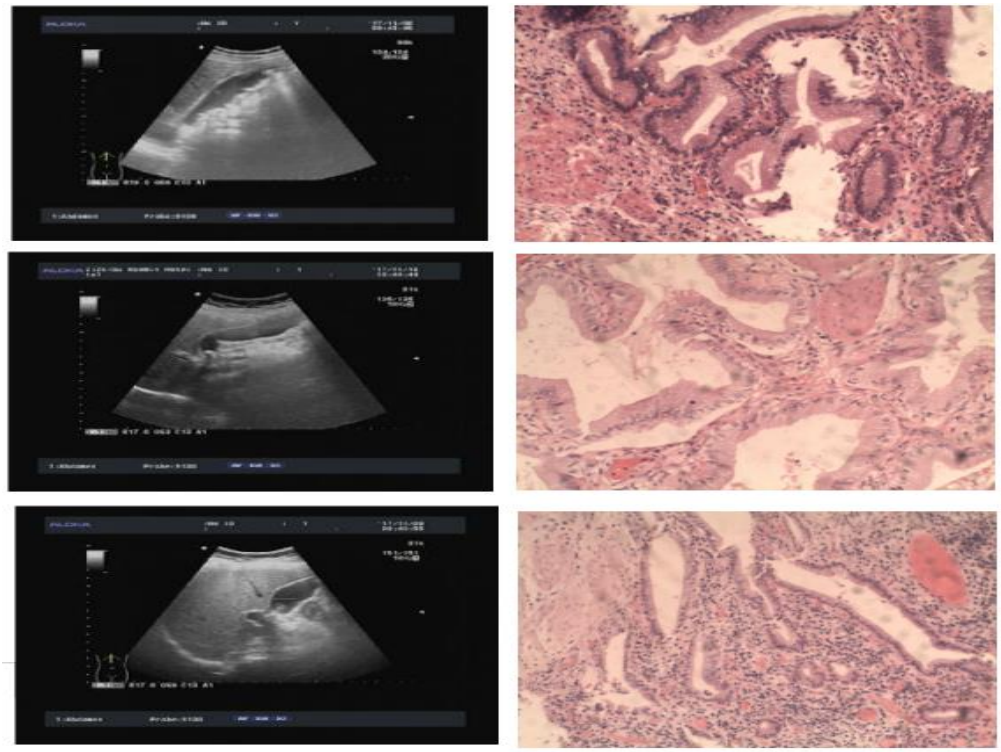

Figure 1. **Imaging and pathological diagnosis of 3 patients with calculous cholecystitis.**
A. abdominal B-ultrasound image of No.75076. It shows focal lesions and may have hemangioma. **B.** pathological section image of No.75076. Gallbladder stones can be diagnosed, and no space-occupying lesions are found in the pancreas, kidneys and spleen. **C.** abdominal B-ultrasound image of No.75508. It shows possible concurrent (mild) fatty liver. **D.** pathological section image of No. 75508. Chronic cholecystitis and gallstones can be diagnosed. In the patient's pancreas, there are no space-occupying lesions in the kidneys, and the spleen is abnormally small. **E.** abdominal B-ultrasound image of No.75133. It shows that there may be fatty liver. **F.** pathological section image of No. 75133. Chronic cholecystitis and gallstones can be diagnosed. In the patient's pancreas, no occupying lesions are seen in the kidneys, and the spleen is abnormally small.The blue represents No.75508, yellow represents No.75133 and green represents No.75076

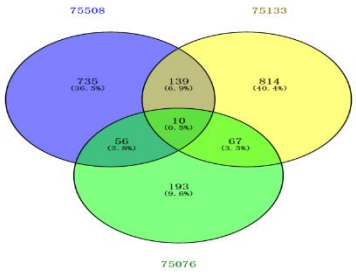

Figure 2**. The distribution of gallbladder tissue-specific expression proteins.**

Mining functional modules in calculous cholecystitis

Based on the STRING database, we identified direct interactors of these tissue-specific proteins and constructed a protein-protein interaction (PPI) network, which may represent potential pathogenesis mechanism of calculous cholecystitis. Moreover, we identified 24 functional modules (Figure 3), referring to 2189 nodes.

Gene Ontology (GO) function enrichment analysis

of 24 modules showed that there were 1329 cell composition (CC) tems, 1061 molecular functional (MF) terms, and 7418 biological processes (BP) terms (Figure 4). We observed that functional modules tend to enrich in multiple disease-related functions, such as regulation of cell-cell adhesion mediated bycadherin, receptor mediated endocytosis involved in cholesterol transport and regulation of inflammatory response. 398 KEGG pathway entries showed that module genes were mainly involved in cell adhesion molecules (CAMs), NF-kappa B signaling pathway and Adipocytokine signaling pathway, which have been shown to be associated with the development and progression of calculous cholecystitis. In view of these functions and pathways, we identified these 24 modules as dysfunctional modules. In addition, we also found that these module genes were also significantly involved in cancer-related functions and pathways, such as positive regulation of tumor necrosis factor production and proteoglycans in cancer., indicating the risk of canceration of calculous cholecystitis. Further, a functional network was constructed with BinGO (Figure 5).

Figure 3. **24 functional modules in calculous cholecystitis.**
Large circles represent proteins within the module, while small circles represent proteins outside the module and are not further analyzed in subsequent studies. Large circles of different colors represent different modules, and 24 colors represent 24 functional modules related to calculous cholecystitis.

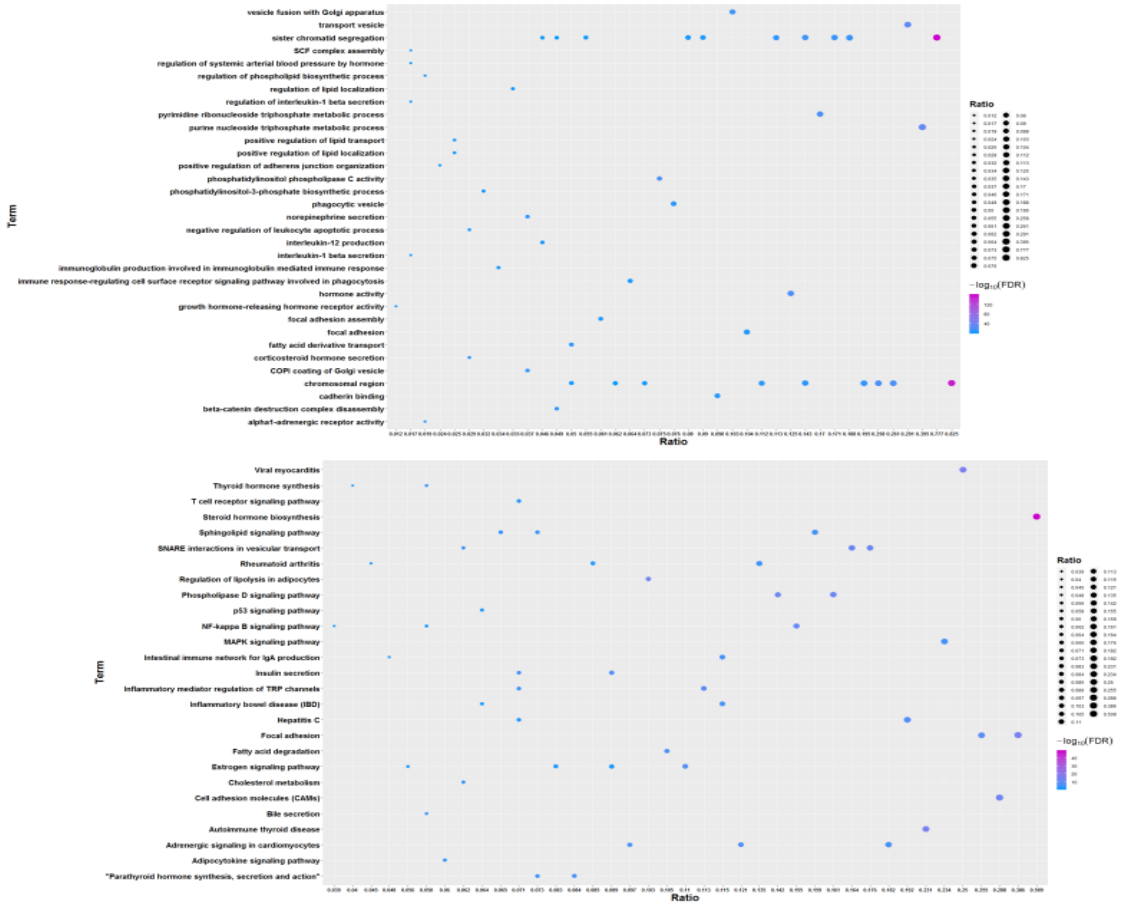

Figure 4. **Function and pathway enrichment analysis of modules.**
A. the results of GO function enrichment analysis. **B.** the results of KEGG pathway enrichment analysis. Color represents significance and the purple is most significant. The size of the circle represents the number of entries genes in module involved in.

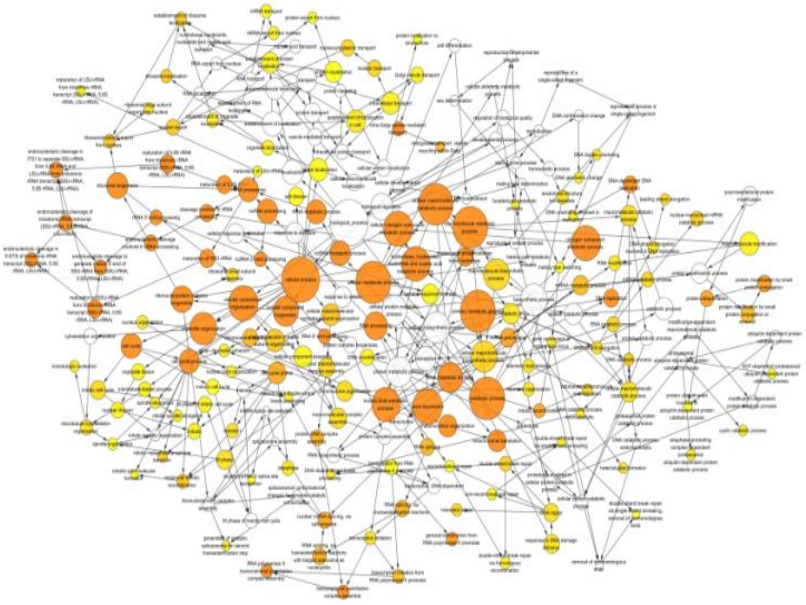

Figure 5**. Analysis of the overall enrichment of the module gene.**
The deeper the color, the more significant the enrichment. The size of the circle represents the number of entries genes in module involved in.

Identifying pivot regulators of the dysfunctional modules

Although the regulatory relationships of transcription factors (TFs) and ncRNAs in calculous cholecystitis have been studied, little attention has been paid to their comprehensive regulation of dysfunctional modules. To explore these regulators, we explored pivot regulators of dysfunction modules based on transcriptional and post-transcriptional regulatory relationships. Based on the number of interactions and the significance, we identified a total of 1488 pivot regulators (Figure 6), including 83 TFs, 104 TF-target pairs and 1405 ncRNAs, 2199 ncRNA-associated interactions. Statistical analysis found that TF E2F1 has a significant regulatory effect on four dysfunctional modules. MYC, NFKB1 and TP53 have a significant regulatory effect on three modules. It suggested these TFs may mediate dysfunctional modules to regulate the occurrence and development of calculous cholecystitis and play a key role in the pathogenesis. In addition, targets of long non-coding RNA (lncRNA) CRNDE and miR-590-3p were up to 11 dysfunctional modules. FENDRR and miR-340-5p regulated 10 dysfunctional modules, which means they have potentially important regulatory effects on calculous cholecystitis. In general, an in-depth study of the regulation of these pivot regulators on dysfunctional modules will help us fully understand the underlying mechanisms of disease. These pivot regulators can also be used as candidates for further experimental studies by other biologists.

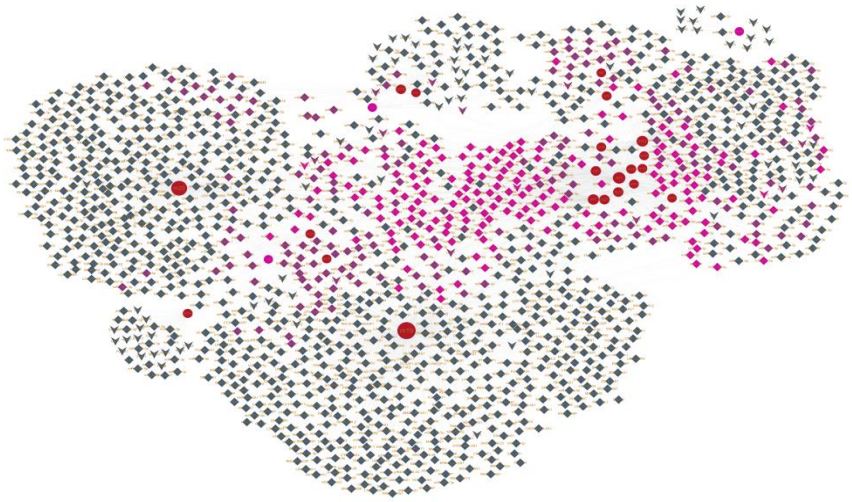

Figure 6. **Pivot regulators-mediated regulation network.**Color represents the degree of node and the red is greatest. The circle represents the module, the diamond represents ncRNA and the arrow represents TF.

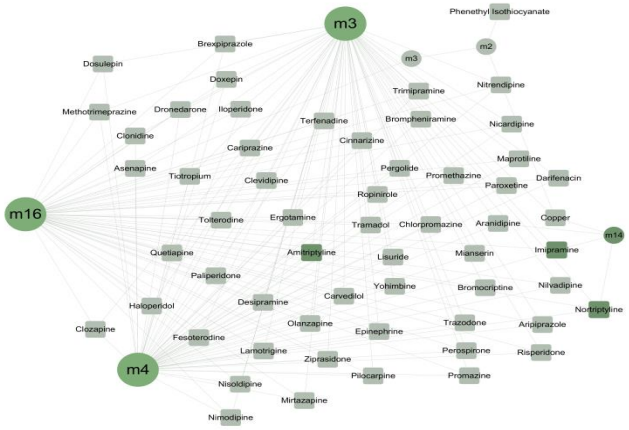

Figure 7. **Regulation relationships between potential drugs and modules.**
Green to gray represents the degree of the node from large too small. Circles represent modules and squares represent potential drugs. This figure only shows drugs that regulate 3 or more modules.

Exploration of potential drugs for gallstones with

cholecystitis

A comprehensive and in-depth understanding of the molecular mechanisms is aimed at exploring its potential therapeutic mechanisms and exploring effective treatments. Here, based on the relationships between the calculus cholecystitis dysfunction modules and the drug-target relationships, we conducted a prediction of the potential drugs. It was found that 904 drugs and 1170 drug-target pairs, which may represent their potential therapeutic mechanisms (Figure 7). Statistical analysis showed that trace element copper had significant pharmacological or toxic effects on five dysfunctional modules. Amitriptyline, Imipramine and Nortriptyline produced potential therapeutic or side-effect effects on four modules. Other drugs also targeted dysfunction module, which has certain curative effect or toxic effect on calculous cholecystitis. These results requireed further research and exploration. These potential drugs for calculous cholecystitis provided a reference and inspiration for pharmacobiologists to conduct pharmacological and side-effect analysis, and can be used as drug candidates for drug relocation studies. The potential drug prediction for dysfunctional modules in disease has also become an important research method for personalized treatment and personalized medicine.

Discussion

Gallstones is one of the common biliary system diseases, which can lead to a variety of complications, including cholecystitis, choledocholithiasis, gallstone ileus and gallstone pancreatitis (Schirmer et al, 2005). The complex relationship between gallstones and cholecystitis is particularly significant, and the two appear together as a risk factor for each other, representing a large overlap between molecular mechanisms and physiological levels. Although the close relationship between gallstones and cholecystitis has been generally accepted, it still needs a large-scale exploration based on functional gallstone pancreatitis modules. We integrated tissue-specific expression proteins in the gallbladder of three patients to study the underlying pathogenesis of calculous cholecystitis. According to enrichment analysis and pivot regulators analysis, we found that dysfunctional modules activated by pivot TFs and ncRNAs which can mediate a range of functions and pathways and regulating the occurrence and development of calculous cholecystitis. The enrichment analysis results showed that the module genes mainly participated in immune responses, such as innate immune response activating cell surface receptor signaling pathway, regulation of leukocyte cell-cell adhesion and T cell receptor signaling pathway. Studies have shown that T cells not only play a key role in cholesterol production, but also that cholesterol monohydrate crystals induce the expression of pro-inflammatory cytokines in a T cell-dependent manner. Therefore, acquired immunity and inflammation are not only the result of cholelithiasis, but also key factors in the pathogenesis of cholesterol stones (Maurer et al, 2007). In addition, the module genes were also found to be involved in the regulation of cellular response to insulin stimulus, which consistent with the findings of *Fang BJ* et al.that multiple aspects of the insulin signaling pathway are associated with gallstone formation(Fang et al, 2016). It is noteworthy that up to 10 modules significantly enriched to the cancer-related functions and pathways, such as positive regulation of tumor necrosis factor superfamily cytokine production and proteoglycans in cancer. The results of this analysis were verified by Gadzhiev *DN* et al. (Gadzhiev et al, 2013). Multiple dysfunctional modules were involved in cancer-related functions and pathways, suggesting that persistent chronic cholecystitis caused by gallstones may be an important risk factor for various cancers, especially gallbladder cance(Yildiz et al, 2015; Andreotti et al, 2011; Roa et al, 2006). Based on the results of these enrichment analyses, we can observe that each module participates in the function and pathway of calculous cholecystitis to varying degrees and therefore it is identified as a disease dysfunctional module. In-depth study of these dysfunctional modules helps us further understand the underlying mechanisms of calculous cholecystitis.

Transcriptional and post-transcriptional regulation have always been considered as key factors in the occurrence of disease. To elucidate the transcriptional regulatory factors of calculous cholecystitis, we identified pivot regulators for dysfunction modules based on transcriptional and post-transcriptional regulatory relationships. It was found that long non-coding RNAs CRNDE and FENDRR, miR-590-3p and miR-340-5p, and transcription factors E2F1, MYC, NFKB1 and TP53significantly regulated dysfunctional modules. Colorectal neoplasia differentially expressed (CRNDE) can regulates a number of inflammatory and cancer-related pathways. It can act as a scaffold for DMBT1 and C-IAP1 complexes to activate the PI3K-AKT pathway, which promoting the occurrence of gallbladder cancer (Shen et al, 2016). CRNDE strongly promotes cell proliferation of renal cell carcinoma (RCC) by activating the Wnt/β-catenin

signaling pathway (Shao et al, 2016). When it is regulated by insulin/IGF signal, it can promote the metabolic changes of cancer cells to aerobic glycolysis (Warburg effect), which may be related to colorectal cancer and regulate genes involved in central metabolism (Ellis et al, 2014). In addition, it is associated with the occurrence, development and prognosis of cancers, such as glioma, ovarian cancer, hepatocellular carcinoma and pancreatic cancer, and is a typical pan-cancerous-associated lncRNA(Wang lli et al, 2015; Szafron et al, 2015; Chen et al, 2016; Wang et al, 2017). This may be a potential mechanism of various cancers which induced by persistent chronic inflammatory stimuli due to gallstones. FENDRR is also associated with the progression, metastasis and prognosis of various cancers, which regulates the metastasis of gastric cancer cells by affecting the expression of fibronectin-1, and thus its reduced expression is associated with poor prognosis of gastric cancer (Xu et al, 2014). MiR-590 is involved in many inflammations, sclerosis and cancer-related functions and pathways. It inhibits the inflammation and cancer molecular signaling pathways in breast cancer cell lines MDA-MB-231 and MCF-7 and has been identified as a good candidate target for breast cancer treatment (Sheikholeslami et al, 2017). It attenuates lipid accumulation and proinflammatory cytokine secretion by targeting lipoprotein lipase gene in human THP-1 macrophages and is therefore considered as a potential strategy for the treatment of atherosclerotic cardiovascular disease (He et al, 2014). In addition, the DICER1-miR-590-5p-YAP1 axis in colorectal cancer can inhibit tumorigenesis and affect patient survival, which guides the exploration of colorectal cancer diagnostic biomarkers and targeted therapeutic molecules (Ou et al, 2017). Both lncRNAs and miRNAs, which regulating multiple modules, targeted genes and pathways related to inflammation and cancer, suggesting that gallstones can not only induce inflammation such as cholecystitis, but also have a high probability of causing cell carcinogenesis. On the other hand, the transcription factors E2F1 and NFKB act as potential therapeutic targets and key mediators of inflammation-associated cancers, and their interactions can regulate inflammation and metabolism in cells, driving inflammatory cytokines and immune responses in the tumor microenvironment (Huang et al, 2016; Palomer et al, 2011). In the process of gallstone-induced inflammation, these two transcription factors

mediated four and three dysfunction modules, respectively, playing an important role in the pathogenesis of calculous cholecystitis and even gallbladder cancer. In addition, down-regulation of c-myc mRNA and protein expression can effectively inhibit the proliferation of bile duct epithelium, submucosal gland and collagen fibers, significantly inhibit chronic proliferative cholangitis (CPC), and reduce calculus complications (Li et al, 2009). TP53 has been confirmed by several studies that play a different role in the sequential pathogenesis of gallstones, chronic cholecystitis and gallbladder epithelial dysplasia into gallbladder carcinoma (GBC) (Moreno et al, 2005). Specifically, sporadic TP53 early-switching mutations in cholecystitis mediate cell turnover, increased oxidative stress, cell cycle dysregulation, apoptosis and replicative senescence (Yanagisawa et al, 2010; Espinoza et al, 2016). It can be considered that the change of the transcription factor TP53 can represent a pathway from gallstone to cholecystitis and then to gallbladder cancer. And modules 12, 17, 22 regulated by TP53 may be an important dysfunction mechanism to achieve this pathway. These pivot regulators collectively mediated dysfunctional modules and played a global regulatory role in characterizing the underlying pathogenic mechanisms of calculous cholecystitis.

It is well known that drug treatment has always been plagued by drug resistance and drug toxicity. A comprehensive and systematic understanding of the molecular mechanisms of the pathogenesis of calculous cholecystitis is the key to drug development and personalized treatment. The multi-regulator-mediated dysfunctional modules obtained from this work are precisely for the purpose of clearly understanding and exploring the pathogenesis of calculous cholecystitis. Based on these multi-regulators driven dysfunctional modules and drug-target information, we predicted potential drugs for calculous cholecystitis. It was found that copper significantly regulated five dysfunctional modules, which may have a potential effect on calculous cholecystitis. According to *Verma GR* et al., high levels of calcium and trace elements (copper, zinc and iron) in the bile of patients with chronic cholelithiasis result in a slightly alkaline bile acid, which may be a potential cause of gallstones (Verma et al, 2002). Thus, copper may be potentially toxic to calculous cholecystitis and should be taken care of in the patient's daily diet. In addition, three common antidepressants: Amitriptyline, Imipramine and Nortriptyline, were predicted to modulate four dysfunctional modules, respectively. In particular, Nortriptyline is a metabolite of Amitriptyline. Studies have shown that Amitriptyline can increase the dissolution rate of cholesterol in bile, improve

the speed of cholesterol gallstone dissolution therapy and have potential therapeutic efficacy for gallstones (King et al, 1985). However, studies by *Andjelkovic M* et al. suggested that it is wise to avoid using Amitriptyline in patients with cholecystitis because it may inhibit gallbladder emptying (Andjelkovic et al, 2011). Therefore, whether to use Amitriptyline for the treatment of calculous cholecystitis needs further exploration. Whether Imipramine and Nortriptyline have clinical effects on gallstones and cholecystitis has not been studied, and further exploration is needed. Although other drugs regulated less modules, they may also be potential drugs for the treatment of calculous cholecystitis or drugs with toxic side effects, which require further exploration. In a word, it is undoubted that this study provides a new method for predicting potential drugs for the treatment of diseases, which not only helps drug developers to relocate drugs, but also provides a theoretical basis of personalized treatment and dietary guidance for clinical staff. In general, the functional module-based approach not only provides a comprehensive and in-depth exploration of the mechanisms of disease occurrence and development, but also provides a wealth of resources for potential candidate TFs and ncRNAs, as well as exploring its potential treatments and therapeutic mechanisms.

Conflict of interests

The authors declare no conflict of interest.

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