Article

Metabolic pathway of non-alcoholic fatty liver disease: Network properties and robustness

WenJun Zhang, **YuTing Feng**
School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, China; International Academy of Ecology and Environmental Sciences, Hong Kong
E-mail: zhwj@mail.sysu.edu.cn, wjzhang@iaees.org

Received 1 December 2016; *Accepted 5 January 2017*; *Published 1 March 2017*

Abstract
Nonalcoholic fatty liver disease (NAFLD) is a systematic and complex disease involving various cytokines/metabolites. In present article, we use methodology of network biology to analyze network properties of NAFLD metabolic pathway. It is found that the metabolic pathway of NAFLD is not a typical complex network with power-law degree distribution, $p(x)=x^{-4.4275}$, $x\geq 5$. There is only one connected component in the metabolic pathway. The calculated cut cytokines/metabolites of the metabolic pathway are SREBP-1c, ChREBP, ObR, AMPK, IRE1α, ROS, PERK, elF2α, ATF4, CHOP, Bim, CASP8, Bid, CxII, Lipogenic enzymes, XBP1, and FFAs. The most important cytokine/metabolite for possible network robustness is FFAs, seconded by TNF-α. It is concluded that FFAs is the most important cytokine/metabolite in the metabolic pathway, seconded by ROS. FFAs, LEP, ACDC, CYP2E1, and Glucose are the only cytokines/metabolites that affect others without influences from other cytokines/metabolites. Finally, the IDs matrix for identifying possible sub-networks/modules is given. However, jointly combining the results of connectedness analysis and sub-networks/modules identification, we hold that there are not significant sub-networks/modules in the pathway.

Keywords non-alcoholic fatty liver disease; network analysis; crucial cytokines/metabolites.

Network Pharmacology
ISSN 2415-1084
URL: http://www.iaees.org/publications/journals/np/online-version.asp
RSS: http://www.iaees.org/publications/journals/np/rss.xml
E-mail: networkpharmacology@iaees.org
Editor-in-Chief: WenJun Zhang
Publisher: International Academy of Ecology and Environmental Sciences

1 Introduction

Nonalcoholic fatty liver disease (NAFLD) is a pathological phenomenon caused by the accumulation of triglycerides (steatosis) and accompanied by or without necrotizing inflammation or fibrosis. Some research argued that NAFLD is a disease that under the condition of daily alcohol intake of less than 10g (or 20g) for woman and less than 30g for man, the liver fat accumulation is 5% greater than the weight of the liver (Bonora and Targher, 2012; Byrne, 2013). The disease is easily induced by excessive alcohol consumption or other known factors such as drug-induced liver disease, viral or autoimmune hepatitis, hemochromatosis or inflammatory bowel disease, etc (Bonora and Targher, 2012). NAFLD includes several pathological cases,

simple steatosis, non-alcoholic steatohepatitis (NASH), and cirrhosis (Targher et al., 2010; Xie et al., 2010; 2011).

NAFLD is a common disease in the human population, with the estimated prevalence of 17% to 33% in common population and 70% in the population of type 2 diabetes mellitus (Byrne, 2012). With the increasing prevalence of obesity and metabolic diseases, the incidence of NAFLD is on the rise around the world (Bonora and Targher, 2012). NAFLD has been suspected to involve in cardiovascular disease and chronic kidney disease (Bonora and Targher, 2012). As a multidimensional metabolic disorder syndrome concerning environment, genetics and spirit stress, the disease may be clinically involved in, from primary care physicians to gastroenterologists, cardiologists, radiologists, and gynecologists (Vuppalanchi and Chalasani, 2009; Targher et al., 2010; Xie et al., 2010; Byrne and Targher, 2015).

NAFLD is a systematic and complex disease involving various cytokines/metabolites (Kanehisa Laboratories, 2016). In present article we try to use methodology of network biology (Zhang, 2012a) to analyze network properties of NAFLD metabolic pathway and achieve some useful results for further research and application.

2 Material and Methods

2.1 Data source and pre-processing

Data of metabolic pathway of non-alcoholic fatty liver disease were collected from KEGG-PATHWAY database (Kanehisa Laboratories, 2016; Fig. 1).

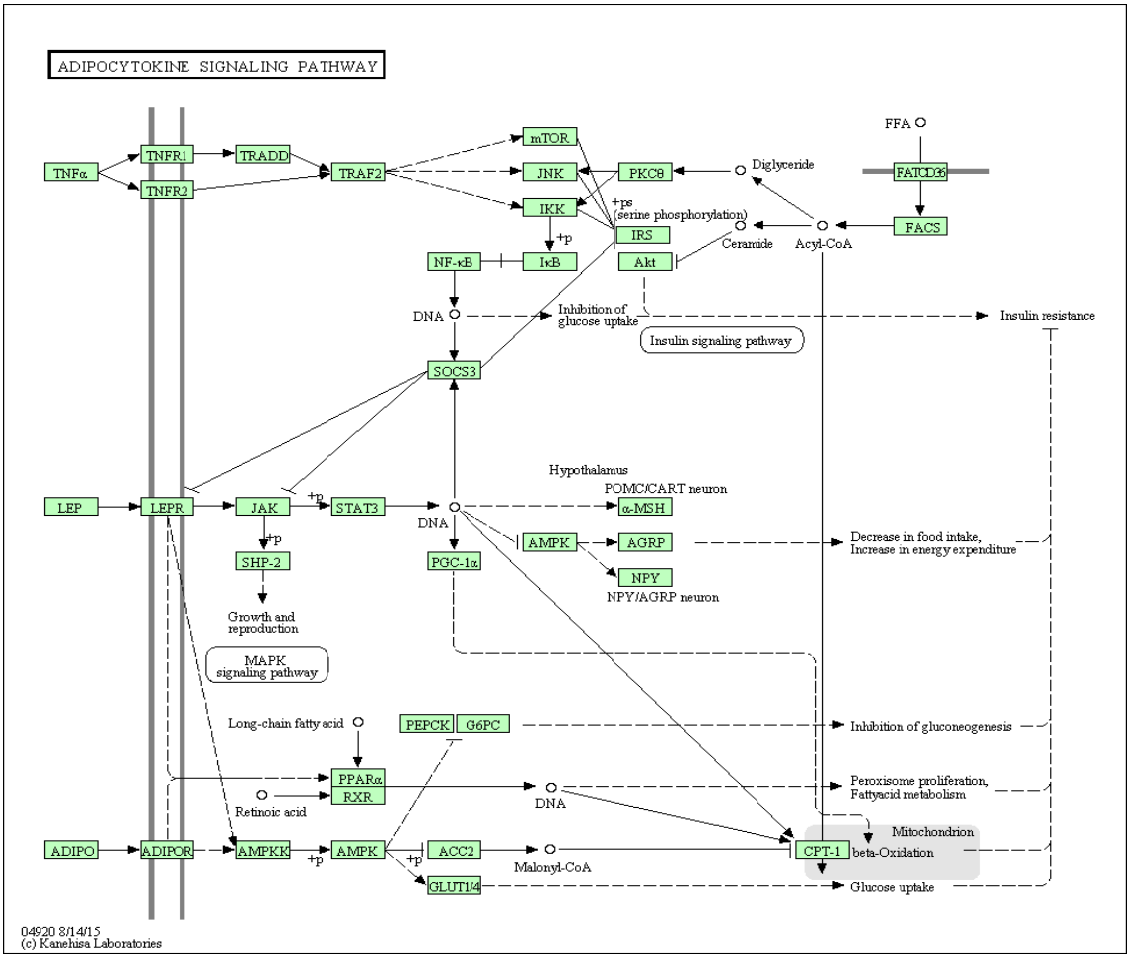


Fig. 1 Metabolic pathway of non-alcoholic fatty liver disease (Kanehisa Laboratories, 2016).

All cytokines/metabolites in the pathway were numbered and links between cytokines/metabolites were labeled. Finally, the adjacent matrix (for directed graph) of metabolic pathway of non-alcoholic fatty liver disease was produced as the following, where the row/column IDs of the matrix are, 1: IL6, 2: IL6R , 3: SOCS3, 4: TNF-α, 5: TNFR1, 6: NF-κB, 7: INS, 8: INSR, 9: IRS1/2, 10: PI3K, 11: Akt, 12: GSK-3, 13: LXR-α, 14: RXR, 15: SREBP-1c, 16: ChREBP, 17: L-PK, 18: LEP, 19: ObR, 20: ACDC, 21: adipoR, 22: AMPK, 23: PPAR-α, 24: Cdc42, 25: Rac1, 26: MLK3, 27: JNK1/2, 28: ASK1, 29: JNK1, 30: AP-1, 31: c-Jun, 32: ITCH, 33: IRE1α, 34: TRAF2, 35: IKKβ, 36: IL1, 37: CYP2E1, 38: ROS, 39: FasL, 40: IL8, 41: TGF-β1, 42: PERK, 43: elF2α, 44: ATF4, 45: CHOP, 46: Bim, 47: Bax, 48: Fas, 49: CASP8, 50: Bid, 51: CxI/II/III/IV, 52: CxII, 53: CxIII, 54: CxIV, 52: Cytc, 53: CASP3, 54: CASP7, 55: Oxysterol, 56: Fatty Acyl-CoA, 57: Lipogenic enzymes, 58: Glucose, 59: XBP1, 60: C/EBPα, and 61: FFAs, respectively. In the matrix, 1 and -1 represent positively and reversely directed link between two cytokines/metabolites respectively, and 0 means no link.

0 1 0 0 0 -1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
-1 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 -1 0 0 0 0 0 0 1 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 1 -1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 -1 -1 0 0 0 0 0 0 -1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1
0 0 0 -1 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
1 0 0 1 -1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 1 0 0 0 -1 0 0 0 0 0 0 0 0 0 -1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 -1 0 1 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0
0 0 -1 0 0 0 0 -1 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 -1 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 -1 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 1 0 0 0 -1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 -1 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 -1 0 1 0 0 0 0 0 0 0 -1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 -1 0 0 0 0 0 0 0 0 0 0 -1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 -1 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 -1 0 -1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 -1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 -1 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 -1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 1 0 0 0 0 -1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 0 -1 0 1 1 0 0 0 0 0 0 -1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
1 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 1 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 -1
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 -1 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 0 0 0 -1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 -1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 -1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 1 0 -1 0 1 1 1 0 0 0 0 0 0 0 0 0 -1 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 -1
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0

0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 0 1 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 0 0 1 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 0 0 1 1 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 -1 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 -1
0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 -1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -1 0 0
0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 1 0 0 0 0 0 0 0 1 0 0 0 0 0 1 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0

In the calculation of network type and centrality measures below, every -1 in the adjacency matrix above are transformed to 1, i.e., the adjacency matrix of undirected network is used.

2.2 Methods

2.2.1 Network type

In present study, in addition to power law distribution, binomial distribution, Poisson distribution, and exponential distribution, more indices are used to determine network type (Zhang and Zhan, 2011; Zhang, 2012a):

(1) Coefficient of variation (*H*). If $H\leq 1$ the network is a random network. It is a complex network if $H>1$. Network complexity increases with *H*.

(2) Entropy of network (*E*) A more complex network has a larger entropy. If $E\leq 0$ the network is a random network, and it is a complex network if $E>0$.

(3) Aggregation index (*H*). If $H\leq 1$ the network is a random network. It is a complex network if $H>1$. Network complexity increases with *H*.

(4) Skewness. This index is used to measure the degree of skewness of a degree distribution relative to a symmetric distribution. The smaller the skewness is, the more complex the network is.

2.2.2 Connectedness

Two nodes in a network are connected if there is a path between the two nodes. In a connected network, each pair of nodes is connected (Zhang, 2012a, 2016d).

2.2.3 Cut nodes

A connected network (graph) *X* is a block, if and only if for any three vertices *u*, *v* and *w* in *X*, there exists a path from *u* to *w* and the path does not contain *v* (Chan et al., 2012; Zhang, 2016e). There is not any bottleneck in a block. The node *v* is a bottleneck if any path from *u* to *w* must go through *v*. In this case, *v* is a cut node. Lose of a cut node will lead to disconnection of connected blocks. Therefore, cut nodes are crucial nodes of a network (Zhang, 2012a, 2016e).

2.2.4 Centrality

We calculate the following centrality indices of nodes, which represent the topological properties of a network (Freeman, 1978; Opsahl et al., 2010; Kuang and Zhang, 2011; Zhang and Zhan, 2011; Huang and Zhang, 2012; Zhang, 2012a, 2012b, 2016a, 2016b; Shams and Khansari, 2014; Khansari et al., 2016):

(1) Degree centrality. This is a local centrality based on neighbourhood, which reflects the node influence on its neighbourhoods. The degree centrality of a node is the sum of the weights of the links attached to the node. Degree centrality represents the whole involvement of a node in the network. In-degree and out-degree can be separately calculated and analyzed for nodes in directed networks.

(2) Closeness centrality. This is a distance-based measure. For a distance-based measure, different values can be assigned to different links in weighted networks. Closeness centrality is a global centrality, which represents the independence of a node in the network. Closeness centrality is defined as reciprocal of the sum of the node's geodesic distances to all other nodes in the network.

(3) Betweenness centrality. This is a distance-based measure. It represents the node's ability to control the data flow in the network. Betweenness centrality is the proportion of number of geodesic paths that pass through the given node to total number of geodesic paths between any pair of nodes in the network.

2.2.5 Robustness

There are various indices and methods to represent robustness (Zhang, 2016a). Here we use a simple method to estimate the robustness of metabolic pathway. First, we chose several cytokines/metabolites with higher node degree, i.e., crucial nodes in terms of degree centrality. Remove a cytokine/metabolite, and count the number of its downstream links and nodes

2.2.6 Sub-networks/modules

The method of Zhang (2016f) was used to identify possible sub-networks/modules.

In addition, Pajek was used to generate network diagrams.

3 Results

According to Fig. 2, the left-most nodes are mainly start cytokines/metabolites, and the right-most nodes are mainly termination cytokines/metabolites of the pathway, which represent the cytokines/metabolites that ultimately produce further physiological effects of the disease. Most nodes act as the cytokines/metabolites of start+intermediate+termination.

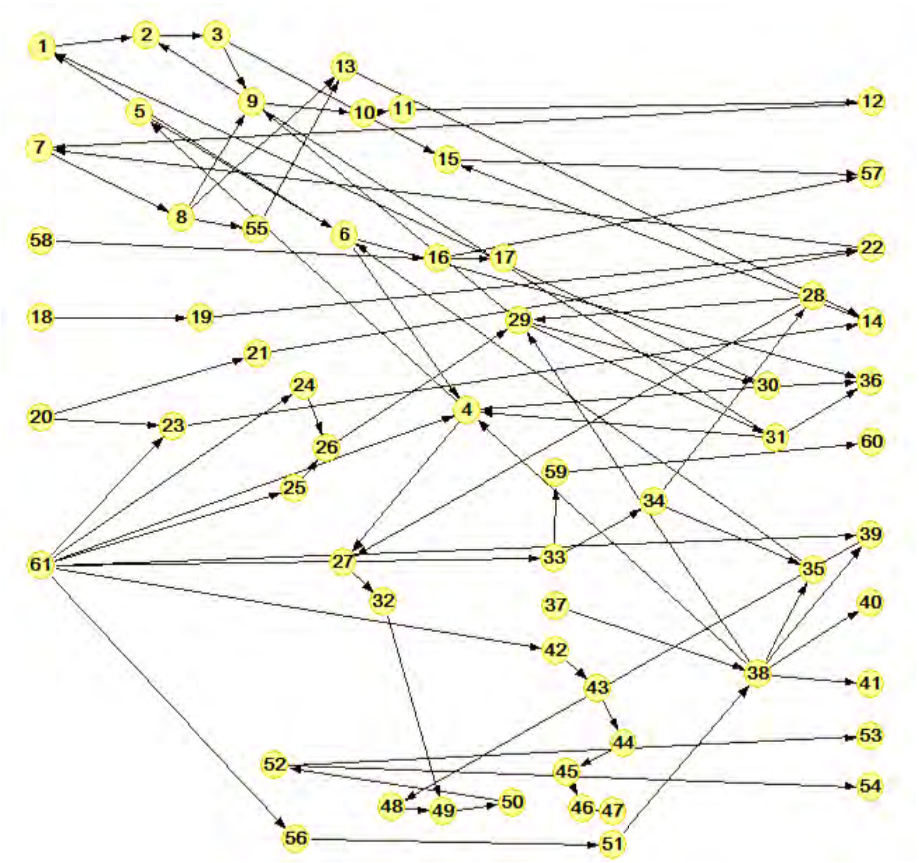


Fig. 2 Network diagram of metabolic pathway of non-alcoholic fatty liver disease.

3.1 Network type

Using revised algorithm of Zhang and Zhan (2011), as shown above, we get the results of determination of network type based on various indices and models as follows

(1) Indices

Skewness of degree distribution: 0.67908.

Aggregation index of the network: 0.94927.

Variation coefficient *H* of the network: 0.8622.

Entropy *E* of the network: -0.37049.

Conclusion: It is a random network.

(2) Degree distributions

(a) Binomial distribution χ^2=5.9411; binomial *p*=0.24317.

Conclusion: Node degrees are binomially distributed. It is a random network.

(b) Poisson distribution χ^2=117.7802; Poisson distribution λ=2.6885.

Conclusion: It is likely not a random network.

(c) Exponential distribution χ^2=82.7677; exponential distribution λ=0.37195.

Conclusion: It is not an exponentially distributed network.

(d) Power-law distribution Kolmogorov-Smirnov goodness-of-fit statistic *D*=0.28439.

Conclusion: Node degrees are power-law distributed, and it is a scale-free complex network.

Power-law α=4.4275; Power-law x_{min}=5.

Power-law distribution density function: $p(x)=x^{-4.4275}$, $x\geq 5$.

According to the comprehensive results above, the metabolic pathway of non-alcoholic fatty liver disease seems to be not a typical complex network.

3.2 Connectedness

Using the algorithm for connectedness (Zhang, 2012a, 2016d), it is found that there is only one connected component in the metabolic pathway of non-alcoholic fatty liver disease.

3.3 Cut nodes

According to the algorithm (Zhang, 2016e), the calculated cut nodes of the metabolic pathway are SREBP-1c (15), ChREBP (16), ObR (19), AMPK (22), IRE1α (33), ROS (38), PERK (42), elF2α (43), ATF4 (44), CHOP (45), Bim (46), CASP8 (49), Bid (50), CxII (52), Lipogenic enzymes (57), XBP1 (59), and FFAs (61).

3.4 Centrality

We use the centrality measures above to calculate the node centrality for metabolic pathway of non-alcoholic fatty liver disease. The results are listed in Table 1. From Table 1, we get the mean ranking of three centrality measures for 61 cytokines/metabolites, as indicated in Table 2. In addition, in-degree, out-degree and their minors of cytokines/metabolites are indicated in Table 3.

From Tables 1-3, it is concluded that FFAs (61) is the most important cytokine/metabolite in the metabolic pathway, seconded by ROS (38). FFAs (61), LEP (18), ACDC (20), CYP2E1 (37), and Glucose (58) are the only cytokines/metabolites that affect others without influences from other cytokines/metabolites.

A higher value of out-degree minusing by in-degree means that the cytokine/metabolite exerts greater influence to other cytokines/metabolites compared with the influence it receives. FFAs (61), LEP (18), INSR (8), ACDC (20), AP-1 (30), and c-Jun (31), etc., are such cytokines/metabolites in the pathway.

Table 1 Three centralities of cytokines/metabolites in metabolic pathway of non-alcoholic fatty liver disease.

Cytokines/ metabolites	Degree	Closeness	Betweenness	Cytokines/ metabolites	Degree	Closeness	Betweenness
1	3	0.0038	0.0393	32	2	0.0035	0.0639
2	3	0.004	0.0486	33	3	0.0042	0.1164
3	3	0.0039	0.0945	34	3	0.004	0.0503
4	7	0.0049	0.1721	35	3	0.0039	0.0519
5	2	0.0039	0.0328	36	3	0.0037	0.0344
6	5	0.004	0.0448	37	1	0.0037	0.0328
7	3	0.0033	0.0896	38	8	0.0047	0.2322
8	4	0.0038	0.1137	39	3	0.0046	0.2339
9	4	0.0043	0.2224	40	1	0.0037	0.0328
10	2	0.0035	0.077	41	1	0.0037	0.0328
11	2	0.003	0.053	42	2	0.0041	0.1831
12	2	0.0028	0.0399	43	2	0.0034	0.1552
13	3	0.0037	0.0672	44	2	0.0029	0.1262
14	3	0.0041	0.177	45	2	0.0025	0.0962
15	3	0.0038	0.1694	46	2	0.0022	0.065
16	3	0.0027	0.0967	47	1	0.0019	0.0328
17	1	0.0023	0.0328	48	2	0.0038	0.1508
18	1	0.0023	0.0328	49	3	0.0034	0.1574
19	2	0.0026	0.065	50	2	0.0028	0.1262
20	2	0.0038	0.1235	51	2	0.004	0.0377
21	2	0.0033	0.1022	52	3	0.0025	0.0967
22	3	0.0031	0.1175	53	1	0.0022	0.0328
23	3	0.0046	0.2743	54	1	0.0022	0.0328
24	2	0.0042	0.0328	55	2	0.0033	0.0328
25	2	0.0042	0.0448	56	2	0.0041	0.0464
26	3	0.0041	0.0459	57	2	0.0031	0.1262
27	3	0.0042	0.0902	58	1	0.0023	0.0328
28	3	0.0043	0.0683	59	2	0.0034	0.065
29	6	0.0047	0.2426	60	1	0.0028	0.0328
30	4	0.0044	0.0546	61	8	0.0052	0.477
31	4	0.0045	0.0776				

1: IL6, 2: IL6R , 3: SOCS3, 4: TNF-α, 5: TNFR1, 6: NF-κB, 7: INS, 8: INSR, 9: IRS1/2, 10: PI3K, 11: Akt, 12: GSK-3, 13: LXR-α, 14: RXR, 15: SREBP-1c, 16: ChREBP, 17: L-PK, 18: LEP, 19: ObR, 20: ACDC, 21: adipoR, 22: AMPK, 23: PPAR-α, 24: Cdc42, 25: Rac1, 26: MLK3, 27: JNK1/2, 28: ASK1, 29: JNK1, 30: AP-1, 31: c-Jun, 32: ITCH, 33: IRE1α, 34: TRAF2, 35: IKKβ, 36: IL1, 37: CYP2E1, 38: ROS, 39: FasL, 40: IL8, 41: TGF-β1, 42: PERK, 43: elF2α, 44: ATF4, 45: CHOP, 46: Bim, 47: Bax, 48: Fas, 49: CASP8, 50: Bid, 51: CxI/II/III/IV, 52: CxII, 53: CxIII, 54: CxIV, 52: Cytc, 53: CASP3, 54: CASP7, 55: Oxysterol, 56: Fatty Acyl-CoA, 57: Lipogenic enzymes, 58: Glucose, 59: XBP1, 60: C/EBPα, and 61: FFAs, respectively.

Table 2 Mean ranking of three centralities of cytokines/metabolites.

Cytokines/ metabolites	Mean ranking	Cytokines/ metabolites	Mean ranking
61	1.3333	25	31.6667
38	3	10	32
29	3.6667	21	33
4	4.6667	24	33
9	7.3333	44	34.6667
23	8.6667	5	35
39	12.3333	52	35
14	12.6667	36	35.3333
31	14.6667	56	36
8	17.3333	57	36.3333
30	17.3333	32	37

15	18	50	37
33	18	11	38.3333
27	19.3333	51	38.6667
3	20	19	39.6667
28	20.6667	45	40.3333
42	21.3333	59	41
6	22.6667	12	42
2	23.6667	46	45.3333
13	25.3333	37	46
49	25.6667	40	47
26	26	41	48
22	26.6667	55	48.3333
7	27	17	54.3333
20	27	18	55.3333
1	27.6667	60	55.3333
34	28	58	58.3333
35	29	53	58.6667
48	29.3333	47	59.6667
16	30	54	59.6667
43	30.6667		

Table 3 In-degree, out-degree and their minus results.

Cytokines/ metabolites	In-degree	Our-degree	Out-degree -in-degree	Cytokines/ metabolites	In-degree	Our-degree	Out-degree -in-degree
1	2	1	-1	32	1	1	0
2	2	1	-1	33	1	2	1
3	1	2	1	34	1	2	1
4	5	2	-3	35	2	1	-1
5	1	1	0	36	3	0	-3
6	2	3	1	37	0	1	1
7	2	1	-1	38	2	6	4
8	1	3	2	39	2	1	-1
9	3	1	-2	40	1	0	-1
10	1	1	0	41	1	0	-1
11	1	1	0	42	1	1	0
12	1	1	0	43	1	1	0
13	2	1	-1	44	1	1	0
14	2	1	-1	45	1	1	0
15	2	1	-1	46	1	1	0
16	1	2	1	47	1	0	-1
17	1	0	-1	48	1	1	0
18	0	1	1	49	2	1	-1
19	1	1	0	50	1	1	0
20	0	2	2	51	1	1	0
21	1	1	0	52	1	2	1
22	2	1	-1	53	1	0	-1
23	2	1	-1	54	1	0	-1
24	1	1	0	55	1	1	0
25	1	1	0	56	1	1	0
26	2	1	-1	57	2	0	-2
27	2	1	-1	58	0	1	1
28	1	2	1	59	1	1	0
29	3	3	0	60	1	0	-1
30	1	3	2	61	0	8	8
31	1	3	2				

3.5 Robustness

Known there are 61 nodes and 82 links in the metabolic pathway. The possible contributions of some crucial cytokines/metabolites to network robustness are listed in Table 4 (illustrated in Fig. 3 also). It is found from Table 4 that the most important cytokine/metabolite is FFAs, seconded by TNF-α.

Table 4 Possible robustness contribution of crucial cytokines/metabolites.

ROS		FFAs		TNF-α		NF-κB		JNK1/2	
No. cytokines/metabolites to be influenced	No. links to be influenced	No. cytokines/metabolites to be influenced	No. links to be influenced	No. cytokines/metabolites to be influenced	No. links to be influenced	No. cytokines/metabolites to be influenced	No. links to be influenced	No. cytokines/metabolites to be influenced	No. links to be influenced
24 (39%)	28 (34%)	37 (61%)	43 (52%)	24 (39%)	27 (44%)	18 (29%)	18 (22%)	22 (36%)	26 (32%)

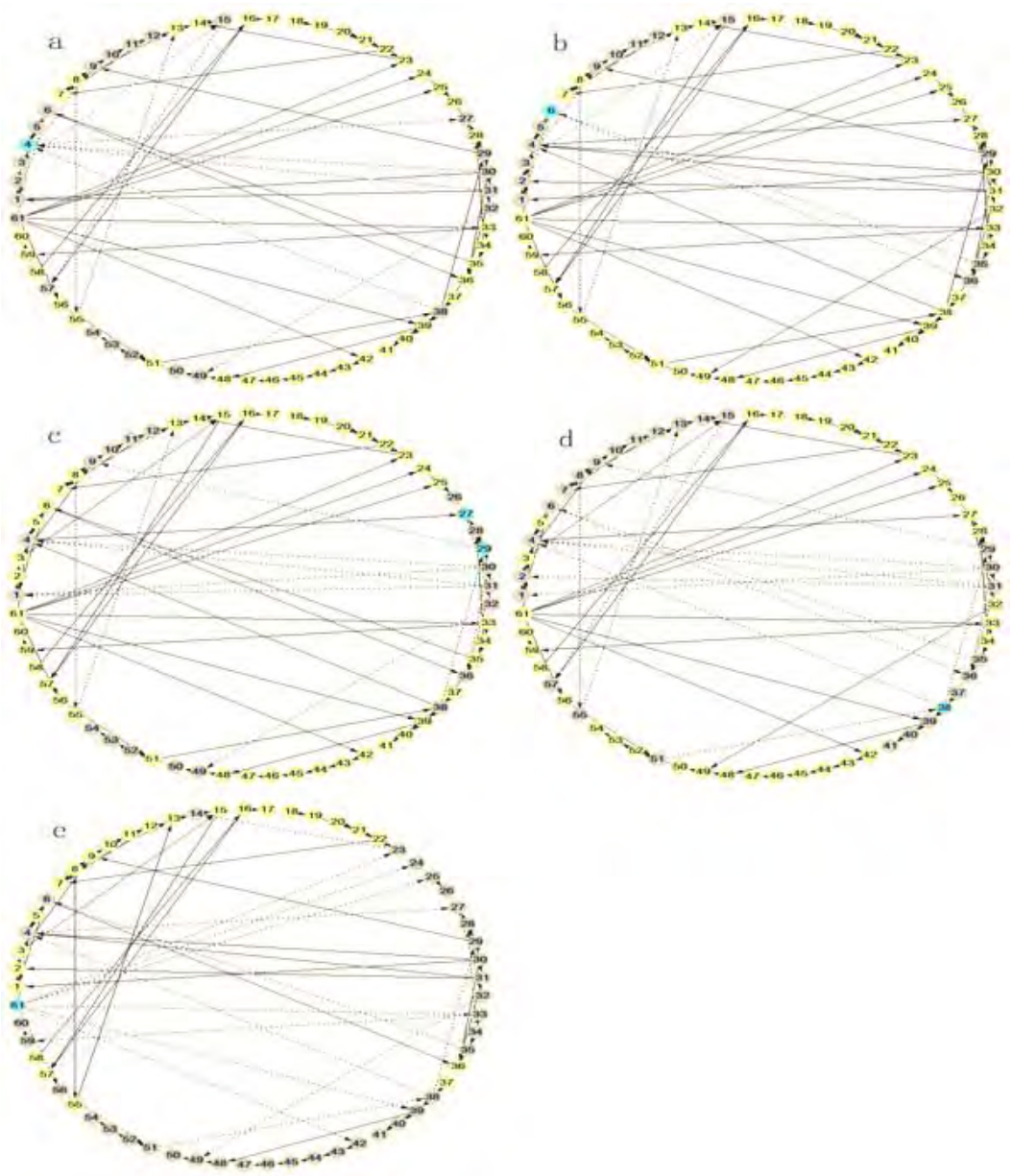
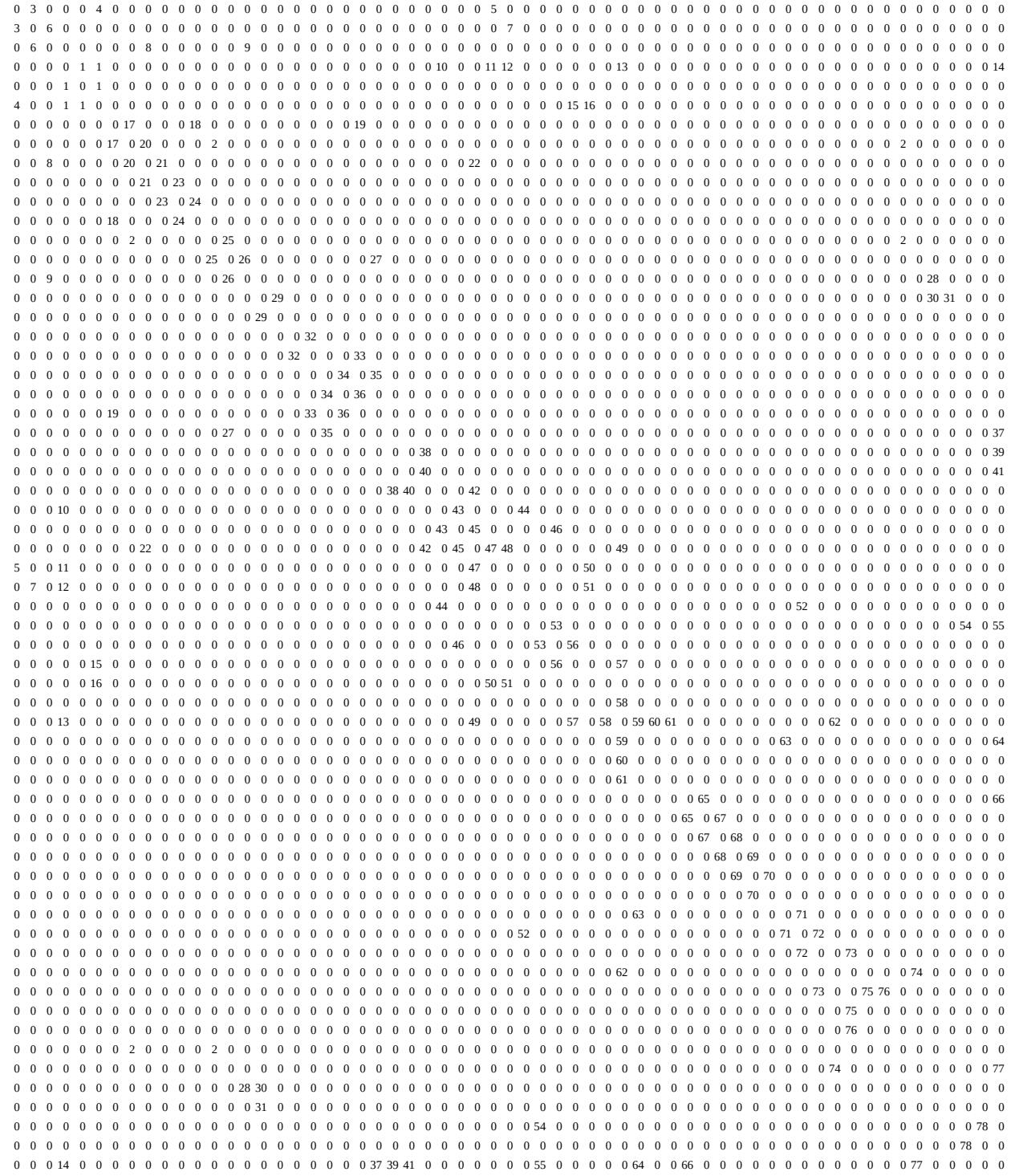


Fig. 3 Illustrations a-e are those after removing TNF-α, JNK1/2, NF-κB, FFAs, and ROS (blue colored nodes), respectively. Gray colored nodes are nodes to be influenced, and dashed lines represent links to be influenced.

3.6 Sub-networks/modules

Using the algorithm of Zhang (2016f), we obtain the IDs matrix for possible sub-networks/modules as the following



Jointly combining the results of connectedness analysis and sub-networks/modules identification, we hold that there are not significant sub-networks/modules in the pathway.

Acknowledgment

We are thankful to the support of Discovery and Crucial Node Analysis of Important Biological and Social Networks (2015.6-2020.6), from Yangling Institute of Modern Agricultural Standardization, High-Quality Textbook *Network Biology* Project for Engineering of Teaching Quality and Teaching Reform of Undergraduate Universities of Guangdong Province (2015.6-2018.6), from Department of Education of Guangdong Province, and Project on Undergraduate Teaching Reform (2015.7-2017.7), from Sun Yat-sen University, China.

References

Bonora E, Targher G. 2012. Increased risk of cardiovascular disease and chronic kidney disease in NAFLD. Nature Reviews Gastroenteroloy and Hepatology, 9: 372-381

Byrne CD. 2012. Dorothy Hodgkin Lecture 2012: non-alcoholic fatty liver disease, insulin resistance and ectopic fat: a new problem in diabetes management. Diabetic Medicine, 29: 1098-1107

Byrne CD. 2013. Ectopic fat, insulin resistance and non-alcoholic fatty liver disease. Proceedings of the Nutrition Society, 72: 412-419

Byrne CD, Targher G. 2015. NAFLD: a multisystem disease. Journal of Hepatology, 62: S47-64

Chan SB, et al. 1982. Graph Theory and Its Applications. Science Press, Beijing, China

Freeman LC. 1978. Centrality in social networks conceptual clarification. Social Networks, 1: 215-239

Huang JQ, Zhang WJ. 2012. Analysis on degree distribution of tumor signaling networks. Network Biology, 2(3): 95-109

Kanehisa Laboratories. 2016. KEGG. http://www.kegg.jp/kegg/pathway.html

Khansari M, Kaveh A, Heshmati Z, et al. 2016. Centrality measures for immunization of weighted networks. Network Biology, 6(1): 12-27

Li JR, Zhang WJ. 2013. Identification of crucial metabolites/reactions in tumor signaling networks. Network Biology, 3(4): 121-132

Opsahl T, Agneessens F, Skvoretz J. 2010. Node centrality in weighted networks: Generalizing degree and shortest paths. Social Networks, 32(3): 245-251

Shams B, Khansari M. 2014. Using network properties to evaluate targeted immunization algorithms. Network Biology, 4(3): 74-94

Sokal RR, Rohlf FJ. 1995. Biometry: the Principles and Practice of Statistics in Biological Research (3rd Edition). W. H. Freeman and Company, New York, USA

Targher G, Chonchol M, Zoppini G, et al. 2011. Risk of chronic kidney disease in patients with non-alcoholic fatty liver disease: is there a link? Journal of Hepatology, 54: 1020-1029

Targher G, Day CP, Bonora E. 2010. Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. New England Journal of Medicine, 363: 1341-1350

Vuppalanchi R, Chalasani N. 2009. Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis: Selected Practical Issues in Their Evaluation and Management. Hepatology, 49: 306-317

Xie P, Zhou XX, Zhang Q. Pathogenesis and treatment of non-alcoholic fatty liver disease. Journal of Chinese Integrative Medicine, 8(3): 201-209

Zhang WJ. 2011. Constructing ecological interaction networks by correlation analysis: hints from community sampling. Network Biology, 1(2): 81-98

Zhang WJ. 2012a. Computational Ecology: Graphs, Networks and Agent-based Modeling. World Scientific, Singapore

Zhang WJ. 2012b. Several mathematical methods for identifying crucial nodes in networks. Network Biology, 2(4):121-126

Zhang WJ. 2016a. Network robustness: Implication, formulization and exploitation. Network Biology, 6(4): 75-85

Zhang WJ. 2016b. Screening node attributes that significantly influence node centrality in the network. Selforganizology, 3(3): 75-86

Zhang WJ. 2016c. Selforganizology: The Science of Self-Organization. World Scientific, Singapore

Zhang WJ. 2016d. Detecting connectedness of network: A Matlab program and application in tumor pathways and a phylogenic network. Selforganizology, 3(4): 117-120

Zhang WJ. 2016e. How to find cut nodes and bridges in the network? A Matlab program and application in tumor pathways. Network Pharmacology, 1(3): 82-85

Zhang WJ. 2016f. A method for identifying hierarchical sub-networks / modules and weighting network links based on their similarity in sub-network / module affiliation. Network Pharmacology, 1(2): 54-65

Zhang WJ, Zhan CY. 2011. An algorithm for calculation of degree distribution and detection of network type: with application in food webs. Network Biology, 1(3-4):159-170