Peer Review File

Article information: http://dx.doi.org/10.21037/tcr-20-2712

Reviewer Comments

Comment 1): Based on the influence of cytogenetic differences on the prognosis, AML can be divided into low-risk group, intermediate-risk group and high-risk group. What are the different roles of the six key genes in the conclusions of this study at different stages?

Reply 1): We feel great thanks for your professional comments on the manuscript. According to your nice suggestions, we have amended the relevant part in manuscript. Changes in the text: Line 152-156, line 239-244, line 299-302, line 413-415, have been marked on the manuscript in dark red.

Comment 2): Since it is a bioinformatics analysis, it may be more meaningful to increase the role of abnormal expression of miRNA in the drug resistance mechanism of acute myeloid leukemia.

Reply 2): Thank you very much for your valuable advice. I'm pretty sure you're right and you make a good point, but the focus of this study is to explore hub genes in AML and their susceptibility to small molecule drugs. In subsequent studies, our team will focus on the relationship between the abnormal expression of miRNA in AML and drug sensitivity, which will be an extremely innovative study.

Comment 3): There are still some weak points in this paper. In gene analysis, the use of IPA software can be increased to analyze the upstream and downstream signal pathways of genes and the analysis of transcription factors. This is more conducive to support the conclusions of this study.

Reply 3): We sincerely appreciate the valuable comments. In this study, we have explored the signaling pathways that may be involved in the progression of AML, as

shown in Figure 2D. IPA software is a very useful and valuable software. At present, our research team has applied for the use account of IPA software. In the following research, we will use IPA software for signal pathway enrichment analysis.

Comment 4): The drug sensitivity analysis in this study is based on the analysis of AML cell lines, and it is recommended to further verify it in animal experiments.

Reply 4): Thanks for your nice suggestions. Based on AML cell lines, we explored 6 hub genes and their correlation with sensitive drugs to provide a basis for guiding clinical medication. Next, we will verify it through animal experiments, which will be a significant and constructive study.

Changes in the text: Line 403-406, were marked out in green in the manuscript.

Comment 5): What is the important role and significance of the six key genes in the conclusions of this study in promoting the refinement and stratification of acute myeloid leukemia and the improvement of clinical treatment plans?

Reply 5): We sincerely appreciate the valuable comments. The high expression of these 6 hub genes identified by our study in AML patients may indicate the high-risk groups at the level of genetic stratification, which will provide a theoretical basis for the treatment of frontline doctors.

Changes in the text: Line239-244, line299-302, Line413-415, were marked out in red on the manuscript.

Comment 6): The DEG analysis used in this study is based on a comprehensive analysis of clinical case samples. It is a summary and analysis of database results and needs to be further verified in animal experiments and other preclinical trials.

Reply 6): Thank you very much for your valuable and meaningful comments on our manuscript. Our study is based on a comprehensive analysis of clinical case samples, which needs to be further verified in animal experiments and other preclinical trials. Next, we will further verify it through animal experiments and other preclinical trial, which will be a significant and constructive study.

Changes in the text: Line403-406, was marked out in green in the manuscript.

Comment 7): The expression verification of key genes is missing in this study. It can be verified by real-time quantitative PCR or immunofluorescence.

Reply 7): We would like to thank you for careful and thorough reading of this manuscript and for the thoughtful comments and constructive suggestions, which help to improve the quality of this manuscript. In our study, 6 significantly overexpressed hub genes in AML patients were identified. Then, we downloaded the data of expression levels of these 6 genes in AML patients and normal bone marrow samples from GEO database for comparative analysis of differential expression, as shown in Figure 5, which verified the above conclusions by bioinformatics methods. Further verification of the above conclusions by real-time quantitative PCR or immunofluorescence is necessary. In the following research work, we will conduct further in-depth research on the expression and mechanism of these six hub genes in AML.

Comment 8): The six key genes selected should be further studied and analyzed, using bioinformatics programs to predict the structure of key genes, chromosome location, physical and chemical properties of encoded proteins, subcellular location, protein functional domains, etc., and then explore their functions.

Reply 8): Thank you very much for your valuable suggestions. The structure, chromosome location, physical and chemical properties of encoded proteins, subcellular location and protein functional domains of six key genes selected have been studied by predecessors, which were pointed out appropriately in the manuscript. FLT3, located in 13q12.2,which is an important cytokine receptor，encodes a class III receptor tyrosine kinase and this receptor is activated by binding of the fms-related tyrosine kinase 3 ligand to the extracellular domain, which mainly expresses in the hematopoietic compartment and involved in the apoptosis, proliferation and differentiation of hematopoietic cells. PF4, located in 4q13.3, encoding the protein of

members of the CXC chemokine family. This protein is chemotactic for numerous other cell type and also functions as an inhibitor of hematopoiesis, angiogenesis and T-cell function. The protein also exhibits antimicrobial activity against Plasmodium falciparum.CD163, located in 13q12.2, encodes a protein that clears the receptor cysteine-rich (SRCR) superfamily. It functions as an acute phase-regulated receptor involved in the clearance and endocytosis of hemoglobin/haptoglobin complexes by macrophages, and may thereby protect tissues from free hemoglobin-mediated oxidative damage. This protein may also function as an innate immune sensor for bacteria and inducer of local inflammation. MRC1, located in 10p12.33, a member of the C-type lectin receptor family, encodes the human mannose receptor (MR). It is involved in several biological processes, including cell-cell recognition, serum glycoprotein turnover, and neutralization of pathogens. The protein encoded by this gene is a type I membrane receptor that mediates the endocytosis of glycoproteins by macrophages, and the protein has been shown to bind high-mannose structures on the surface of potentially pathogenic viruses, bacteria, and fungi so that they can be neutralized by phagocytic engulfment. CSF2RB, located in 22q12.3. The protein encoded by CSF2RB is the common beta chain of the high affinity receptor for IL-3, IL-5 and CSF. Defects in this gene have been reported to be associated with protein alveolar proteinosis (PAP). PPBP, located in 10p12.33, is a platelet-derived growth factor that belongs to the CXC chemokine family. This growth factor is a potent chemoattractant and activator of neutrophils. It has been shown to stimulate various cellular processes including DNA synthesis, mitosis, glycolysis, intracellular cAMP accumulation, prostaglandin E2 secretion, and synthesis of hyaluronic acid and sulfated glycosaminoglycan. It also stimulates the formation and secretion of plasminogen activator by synovial cells. The protein also is an antimicrobial protein with bactericidal and antifungal activity. However, their specific functional role in AML is not fully understood. In this study, we found that these six genes play an important role in the progression of AML, and the high expression of these six genes suggests a poor prognosis in AML patients. In the following research, our team will further explore the mechanism of action of these 6 genes in AML.

Changes in the text: Line312-315, line330-333, line346-350, line360-363, line370-372, line378-382, which were marked out in purple in the manuscript.