Reviewer #1:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

1.Specific Comments to Authors: Thank you for the opportunity to review the manuscript titled, Analysis of N6-Methyladenosine-modified mRNAs in Diabetic Cataract. Despite successful surgical replacement with artificial lenses, cataract remains to be one of the leading causes of visual impairment and blindness worldwide. It has been recently suggested that m6A plays a role in DC progression. In this study, authors aimed to investigate the role of altered m6A and differentially expressed mRNAs in DC. This manuscript is well written and preparation. Aiming at study the role of altered M6A and differentially expressed mRNAs in DC, this paper showed abundant data. Finally, the concluded that M6A mRNA modifications may be involved in DC progression via the ferroptosis pathway. To increase the readability, the authors could add a hypothetical pathway diagram related to the role of altered M6A in the progression of DC.

Response : Thanks for your suggestion.

In this study, we postulated that N6-methyladenosine (m6A) could play a crucial role in DC progression, and therein we performed microarray to detect the m6A landscape in DC. At the same time, we analyzed the methylation and differential expression of related mRNA. Furthermore, we also attempt to investigate the potential signaling pathways involved in the pathogenesis of DC using the bioinformatic anaysis methods. As a result, it is found that increased m6A abundance levels were found in the total mRNA of DC samples, and ferroptosis pathways could be participated in the development of DC. Additionally, we found the levels of five methylation-related genes—RBM15, WTAP, ALKBH5, FTO, and YTHDF1—were upregulated in DC samples, which could be associated with the altered m6A abundance.

Since this present study is a preliminary exploration, the specific pathogenesis of m6A's involvement in DC is still unclear. Therefore, we would stduy further in the future to find the specific mechanism and draw the hypothetical pathway diagram related to the role of altered M6A in the progression of DC.

Reviewer #2:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: The original article conducted by Lei Cai and colleagues aimed to investigate the role of altered M6A and differentially expressed mRNAs in diabetic cataract (DC). The authors used multiple methodologies, including epitranscriptomic microarray analyses, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses, and qPCR, to verify their hypothesis. The results showed that M6A abundance level in total mRNA increased in patients with DC, providing new insights into the development of therapeutic strategies for DC. Generally, the topic in this paper is timely and pragmatic, and the manuscript is well-written. As such, I recommend its acceptance after minor revision. The specific comments are listed as below.

1. In this manuscript, microarray analyses of the mRNAs extracted from the lens anterior capsule tissues of the DC and NC samples were performed, showing difference in m6A-methylated mRNAs. This result is the footstone of the article and guided the authors' research. To verify the quality of the microarray data, the authors performed MeRIP-qPCR using four randomly selected mRNAs. My point is how to randomly and evenly select the tested mRNAs? And how to guarantee the representativeness of these mRNAs?

Response : Thanks for your suggestions.

In the present study, we performed microarray analyses that lays the foundation for the following experiments. Therefore, we performed MeRIP-qPCR using four randomly selected mRNAs to verify the quality of the microarray data.

Specifically, we screened the differentially methylated mRNAs under the criteria of p value $\leq$ 0.05 and FC $\geq$ 1.5, and then we selected genes with multiple expression folds for verification with the primers can be designed for those mRNAs. In terms of feasibility, it is unrealistic to verify all the genes to determine the quality of the chip. Therefore, in this study, we randomly selected 4 methylated mRNAs with different fold changes for verification to ensure the reliability of the results to a certain extent.

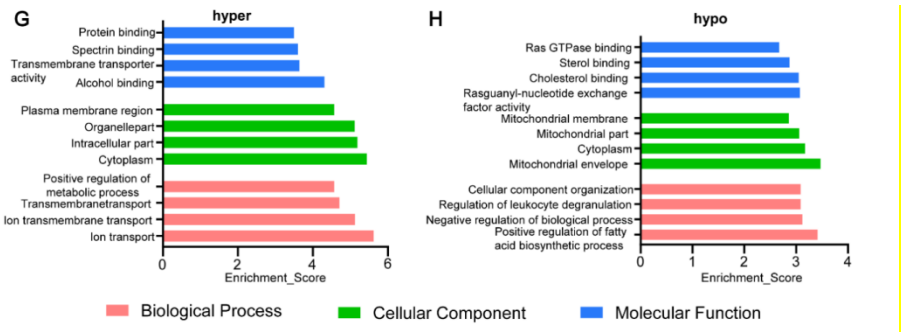
We have modified the contents in the Results section of the manuscript.

2. The authors used GO and KEGG enrichment analyses to explore the biological significance of mRNA M6A modification in DC samples. The enriched GO annotations fond three types of mRNAs: biological process (BP), cellular component (CC), and molecular function (MF). Whereas, the KEGG analysis showed that the mRNAs differentially methylated by M6A participated in 27 pathways. So, what is intersection results of these two analyses? Did the authors conduct the contrastive analysis?

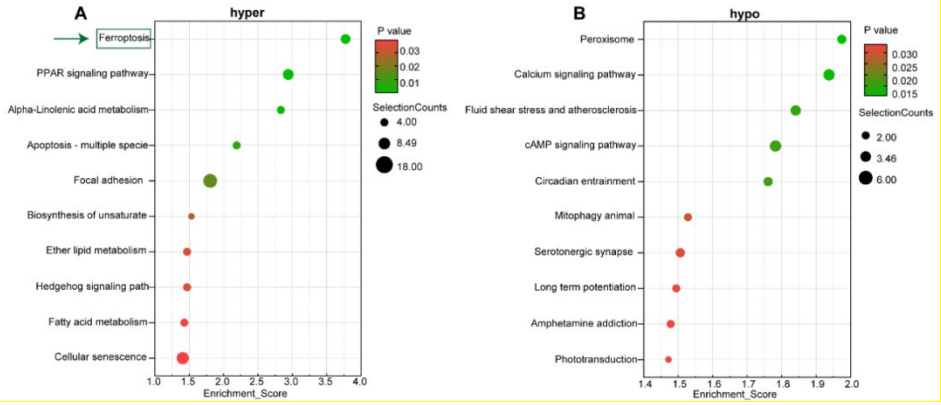
Response : Thanks for your suggestions.

GO analysis and KEGG analysis are two commonly methods in bioinformatics, and they are both used to study gene function. GO analysis mainly relies on lexical search and semantic analysis to help researchers to understand gene functions and biological processes. In contrast, KEGG analysis focuses on finding and analyzing interactions in biological systems. Therefore, GO analysis focuses more on the function of a single gene, while KEGG analysis focuses on the interaction between genes in an organism system.

In our study, the enriched GO annotations were classified into three categories: biological process (BP), cellular component (CC), and molecular function (MF). For the hypermethylated mRNAs, 580 BPs, 110 CCs, and 100 MFs are enriched, and the top four terms with the highest enrichment score are shown in Figure 3G. For the hypomethylated mRNAs, 288 BPs, 47 CCs, and 67 MFs are enriched and the top 4 most enriched terms are shown in Figures 3H.



Furthermore, KEGG pathway analysis showed that the mRNAs differentially methylated by m6A participated in 27 pathways , and the top 10 pathways are shown in Figure 4A－B.



Although the analysis results of GO and KEGG represent different meanings to some extend, it is possible that there is a link between the function of the genes and the pathways involved in the interaction between the genes. For example, the GO results of the hypermethylated mRNAs show that the ion transmembrane transport,ion transport, postive regulation of metabolic process are the potential function of the differentially expressed hypermethylated mRNAs. Meantime, we found that hypermethylated mRNAs were mainly enriched in “ferroptosis”, “PPAR signaling pathway”, and “alpha-linolenic acid metabolism”, which is obviously associated with the GO results.