DR. Ruo-Yu Ma
Scientific Editor
World Journal of Gastroenterology
Email: r.y.ma@wjgnet.com
2019-02-26
Dear DR. Ruo-Yu Ma,

Thank you very much for your and the reviewers' thoughtful evaluations and positive review about our manuscript titled "Analysis of the autophagy gene expression profile of pancreatic cancer based on LC3" (WJG-45490).

In the revision of our manuscript, comments and issues raised by the editors and reviewers have been carefully considered and appropriate changes (highlighted in yellow) have been made. Please find a point-by point response to the reviewers' comments (below). To clarify, we present those requests in *italics* followed by our responses.

We are pleased that the reviewers agree that the manuscript will be a valuable contribution to the literature in this area. We hope that the revised manuscript will now be found acceptable for publication in your journal.

Your prompt consideration of our revision will be greatly appreciated.

Sincerely yours,

Hua Fan, PhD, Academic Fellow
First Affiliated Hospital, College of Clinical Medicine, First Affiliated Hospital, College of Clinical Medicine, Henan University of Science and Technology, No. 24 Jing-Hua Road, Luoyang 471003, Henan, China.
Tel: +86-0379-64830681
Fax: +86-0379-64830681
E-mail: fanhua19851229@haust.edu.cn

Point-by-point response

Response to Reviewer 02742218

Comments To Authors:

The authors have identified deferentially expressed genes involved in the autophagy of pancreatic cancer by a gene expression microarray technique. Protein interaction networks were constructed and the functional clustering of deferentially expressed genes was carried out. Key interacting proteins or genes between modules were screened and evaluated by statistical methods, and the pathogenesis of pancreatic cancer was explored.

Response:

We are very grateful to the Reviewer for his/her recognition of our research work.

Major comment

Over all the study adds to scientific knowledge, ***however, few in vitro experiments on cell lines could further improve the impact of their conclusion.***

Response:

The Reviewer feels that few in vitro experiments on cell lines could further improve the impact of our conclusion. In this study, 347 genes that have no confirmed association with the autophagy process of human pancreatic cancer cells in previous studies were concentrated. Indeed, it is obviously unrealistic to analyze all the genes interacting with LC3 in vitro. Nevertheless, a key gene ubiquitin C which is closely related to the occurrence of perineural invasion (PNI) was determined. Our previous results showed that the high expression of LC3 was positively correlated with PNI in the patients with pancreatic cancer. Suggesting that LC3 may influence the PNI and prognosis of pancreatic cancer through ubiquitin C. Therefore, we have planned to supplement some vitro and vivo experiments to further analysis the relationship between them and explore the molecular mechanism of phagocytosis in pancreatic cancer cells.

Response to Reviewer 03104341

Comments To Authors:

This is a very interesting study. ***However, the authors did not write this manuscript well. Especially, the abstract was written very bad. Of course, there are many english mistakes.***

Response:

We are pleased that the Reviewer feels that the manuscript is interesting. Thank you very much for your kind words and constructive suggestions. In view of we are non-native speakers of English, we have asked professional English language editing companies recommended by your magazine to do the language polishing and provided the language certificate. We have also carefully examined the full text and revised it. The abstract part has also been revised in the revised manuscript and is listing here as well.

ABSTRACT

BACKGROUND

Pancreatic cancer is a highly invasive malignant tumor . Expression levels of the autophagy-related protein microtubule-associated protein 1A/1B-light chain 3 (LC3) and perineural invasion (PNI) are closely related to its occurrence and development. Our previous results showed that the high expression of LC3 was positively correlated with PNI in the patients with pancreatic cancer. In this study, we further searched for differential genes involved in autophagy of pancreatic cancer by gene expression profiling and analyzed their biological functions in pancreatic cancer, which provides a theoretical basis for elucidating the pathophysiological mechanism of autophagy in pancreatic cancer and PNI.

AIM

To identify differentially expressed genes involved in pancreatic cancer autophagy and explore the pathogenesis at the molecular level.

METHODS

Two sets of gene expression profiles of pancreatic cancer/normal tissue (GSE16515 and GSE15471) were collected from the Gene Expression Omnibus (GEO). Significance analysis of microarrays (SAM) algorithm was used to screen differentially expressed genes related to pancreatic cancer. GO analysis and KEGG pathway analysis were used to analyze the functional enrichment of the differentially expressed genes. Protein interaction data containing only differentially expressed genes was downloaded from String database and screened. Module mining was carried out by Cytoscape software and ClusterOne plug-in. The interaction relationship between the modules was analyzed and the pivot nodes between the functional modules were determined according to the information of the functional modules and the data of reliable protein interaction network.

RESULTS

Based on the above two data sets of pancreatic tissue total gene expression, 6098 and 12928 differentially expressed genes were obtained by analysis of genes with higher phenotypic correlation. After extracting the intersection of the two differential gene sets, 4870 genes were determined. GO analysis showed that 14 significant functional items including negative regulation of protein ubiquitination were closely related to autophagy. A total of 986 differentially expressed genes were enriched in these functional items. After eliminating the autophagy related genes of human cancer cells which had been defined, 347 differentially expressed genes were obtained. KEGG pathway analysis showed that the pathways hsa04144 and hsa04020 were related to autophagy. In addition, 65 clustering modules were screened after the protein interaction network was constructed based on String database, and module 32 contains the LC3 gene, which interacts with multiple autophagy-related genes. Moreover, ubiquitin C (UBC) acts as a pivot node in functional modules to connect multiple modules related to pancreatic cancer and autophagy.

CONCLUSION

347 genes associated with autophagy in human pancreatic cancer were concentrated, and a key gene ubiquitin C which is closely related to the occurrence of PNI was determined, suggesting that LC3 may influence the PNI and prognosis of pancreatic cancer through ubiquitin C.

Key words: pancreatic cancer; autophagy-related protein microtubule-associated protein 1A/1B-light chain 3; perineural invasion; GO analysis ; KEGG pathway analysis ; ubiquitin C