Scientific Research Process

Name of Journal: *World Journal of Gastroenterology*

Manuscript NO: 35255

Manuscript Type: BASIC STUDY

Title: Relationship between Pancreatic Cancer Autophagy and Perineural Invasion, Clinicopathological Features and Prognosis

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Dear Editors:

We would like to submit the research article "Relationship between pancreatic cancer cell autophagy and perineural invasion, clinicopathological features and prognosis" to "World Journal of Gastroenterology" for publication. No conflict of interest exits in the submission of this manuscript, and manuscript is approved by all authors for publication. I would like to declare on behalf of my co-authors that the work described was original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part. All the authors listed have approved the manuscript that is enclosed.

World Journal of Gastroenterology as a well-known international journal，the primary aims of the it are to improve diagnostic, therapeutic and preventive modalities and the skills of clinicians and to guide clinical practice in gastroenterology and hepatology.Pancreatic cancer, called “the king of cancer”, is a malignant tumor with poor prognosis. Perineural invasion(PNI)is indicated to be an indicator to predict the recurrence and prognosis of pancreatic cancer after surgery . However, the pathogenesis of PNI has not been defined yet. In this work, we evaluated the relationship between autophagy and PNI in pancreatic cancer cells for the first time. The results of this study can provide a new approach for exploring the mechanism of perineural invasion of pancreatic cancer and improving the prognosis of pancreatic cancer patients. Besides, in view of previous studies of pancreatic cancer there are too few cases of defects, this study were collected from the First Affiliated Hospital of Zhengzhou University hospital , the world's largest hospital, which can provide more research cases, thus to improve the credibility of the study, but also to meet the requirements of World Journal of Gastroenterology influence.

Experimental operation process: (1) First of all ,we consult the postoperative pathology confirmed pancreatic ducta adenocarcinoma cases in the First Affiliated Hospital of Zhengzhou University from 2011.01 to 2016.06, which preliminarily proved that the experiment is feasible. (2) After the approval of the ethics committee of the First Affiliated Hospital of Zhengzhou University, we register the patient's hospital medical record room number and name, to collect relevant patient records, clinical data，such as: age, gender, clinical tumor location, tumor size, tumor differentiation, tumor stage, lymph node metastasis, preoperative diabetes, pancreatitis, preoperative CA199 level, surgery date and so on. (3) Subsequently, we obtained pathological HE staining sections and paraffin blocks of the related patients from the pathology department. pathological sections are reading again by two pathologists according to the principle of double blind, if the results are

inconsistent, intervention by third doctors consultation. Final pathological diagnosis of ductal adenocarcinoma of the pancreas will be included in further studies, otherwise it will be excluded. (4) The tissue sections of the patients were taken and the expressions of UCH and LC3 in the tissues were detected by immunohistochemistry. (5) The patient's survival was recorded by telephone or outpatient treatment. The starting time of follow-up is the time of operation, the termination time is 2016 .08. The primary end point was death from pancreatic cancer. Patients were followed for more than 12 months, and 80 patients were followed for further survival analysis. (6) Data analysis.

Data processing：(1) The immunohistochemical results evaluation were compliance the principle of double blind by two pathologists. If the results are inconsistent, the third pathologists reviewed and negotiated with them.(2) LC3 immunohistochemical score: LC3 immunohistochemistry positive cells required: (1) clear cell structure; (2) located positive particle accurately; (3) pigmentation obviously higher than the background and clear contrast. The positive expression of LC3 was mainly localized in the cytoplasm of pancreatic cancer cells., 5 random powerful visions (400 times) were observed for each case with the optical microscope, and counted 100 homogeneous cells, observed the staining intensity and the proportion of positive cells, and prepared semi quantitative analysis by the product integral method. (1) dyeing intensity: no yellow, 0 points; light yellow 1 points; yellow or deep yellow 2 points; brown or tan 3 points. (2) expression range: <10%, 0 points; 10--25%, 1 points; 26 to 50%, 2 points; 51 to 75%, 3 points; >75%, 4 points. Results of (1) multiplying (2) as the basis: 0 points negative (-); 1 ~ 3 points weakly positive (+); 4 ~ 6 points moderately positive (+ +); 7 ~ 12 points strong positive (+ + +). The results of more than 3 points were high expression, and NMT3 were low expression.**(3)** PNI judging: UCH (ubiquitin carboxy terminal hydrolase) was expressed in the cytoplasm of all nerve fibers. The location of nerve fibers can be clearly defined by UCH, and then determined the invasion of cancer cells to the nerve tissue. The positive PNI judging

standard is that cancer cells are found in the nerve tract, nerve tract, or nerve tract according to precious reports.(4)Survival analysis:The effects of clinicopathological factors such as LC3 expression and perineural invasion on the overall survival rate of pancreatic cancer were analyzed in 80 patients with more than 12 months follow-up. The factors of survival analysis included: the expression of LC3, perineural invasion, age, gender, tumor location, tumor size, histological grade, clinical stage, vascular invasion, lymph node metastasis, pancreatitis, diabetes and preoperative CA199 level.(5)Data analysis:Statistical treatment was performed by SPSS19.0 and GraphPad Prism 5.0 statistical software. Enumeration data were checked by $\chi 2$ test or four grid table Fisher exact probability method. Correlation among clinicopathological factors such as LC3 expression and perineural invasion was analyzed by Spearman correlation method. LC3 and the independent influencing factors of neural infiltration were analyzed by using two categories and unconditional Logistic regression to analyze. Univariate and multivariate analyses were performed on factors that might affect the prognosis by COX risk regression model. Survival curve is plotted by the Kaplan-Meier method. All the results were statistically significant with $P<0.05$.

If the experimental results are inconsistent with previous studies, we will check again if there is any error in our data processing. If there were no mistakes, we would look at the relevant literature and carefully analyze the possible causes of the differences and explain them.

We finally found that perineural invasion of pancreatic cancer cells are independent risk factors for adverse prognosis. There is a significant correlation between them. There must be a pathway between them, through which they interact with each other to promote malignant progression of pancreatic cancer .How to control the role of autophagy in the perineural invasion of pancreatic cancer, and then to improve the progression of cancer still needed for further molecular mechanisms.