Nov. 11, 2016

Dear Editor:

Thank you so much for your letter of November 11, 2016 and the insightful and constructive comments on our manuscript by the expert reviewer. We have revised the manuscript according to these comments. We made the following point by point responses to address the reviewers' comments. The changes in the text are highlighted in yellow.

Comments to the Author(s):

Comments: 1.) Abstract and Introduction should be precise and more logical. Provide the information regarding the importance of cisplatin as a chemotherapeutic drug in gastric cancer treatment and the frequency of occurrence of cisplatin resistance in gastric cancer.

Answer: We would like to thank the reviewer for bringing this important point. We have now included the following discussion in the revised manuscript.

AIM: To explore novel therapeutic target of cisplatin resistance in human gastric cancer.

METHODS: The sensitivity of SGC7901 cells and cisplatin-resistant SGC7901 cells (SGC7901/DDP) for cisplatin were detected by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) assay. High-quality total RNA which isolated from SGC7901/DDP cells and SGC7901 cells were used for mRNA microarray analysis. Results were analyzed bioinformatically to predict their roles in the development of cisplatin resistance and the expression of 13 dysregulated mRNAs we selected were validated by quantitative real-time polymerase chain reaction (qRT-PCR).

RESULTS: SGC7901/DDP cells highly resistant to cisplatin demonstrated by MTT assay. A total of 1308 mRNAs (578 upregulated and 730 downregulated) were differentially expressed (fold change ≥2 and p-values <0.05) in the SGC7901/DDP cells compared with SGC7901 cells. The expression of mRNAs detected by qRT-PCR were consistent with the microarray results. Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and protein-protein interaction (PPI) analysis demonstrated that the differentially expressed mRNAs were enriched in PI3K-Akt, Notch, MAPK , ErbB, Jak-STAT, NF-kappaB signaling pathways which may be involved in cisplatin resistance. Several genes such as PDE3B, VEGFC, IGFBP3,

TLR4, HIPK2 and EGF may associated with drug resistance of gastric cancer cells to cisplatin.

CONCLUSION: Exploration of those altered mRNAs may provide more promising strategy for diagnosis and therapy for gastric cancer with cisplatin resistance.

INTRODUCTION

Gastric cancer is the fourth most common cancer and the second leading cause of cancer death globally [1], and more than two thirds of patients when diagnosed with unresectable disease [2]. The 5-year overall survival rate of patients with advanced gastric cancer approximately 25%[3]. Currently, platinum-based chemotherapy regimen is the standard first-line chemotherapy frequently used for advanced gastric cancer [4, 5], and median overall survival and progression free survival was significantly longer in cisplatin-containing combination therapy compared to non-cisplatin containing regimens[6, 7]. However, cisplatin-based chemotherapeutic agents are often limited in chemotherapy due to drug resistance [8, 9] .

Cisplatin resistance of gastric cancer is multifactorial, accumulating evidence have suggested that the aberrant expression of proteins which associated with decreased cellular accumulation, increased DNA repair capacity, increased drug inactivation [10] play important role in the acquisition of cisplatin resistance. Previous researches have shown that abnormal expression of copper transporter 1 (CTR1) and MRP2 lead to cisplatin resistance by reducing the concentration of cisplatin in cells [11-13]. Moreover, the upregulation of Excision Repair Cross Complementing 1 (ERCC1) [14], X-ray Repair Cross Complementing 1 (XRCC1) [15] and Breast Cancer 1 (BRCA1) [16] have shown to be involved in cisplatin resistance by removal of Pt-DNA adducts [17, 18]. Other studies have shown that downregulation of the human epidermal growth factor receptor II (ErbB2) can significantly enhanced the apoptosis-inducing effects of cisplatin in gastric cancer [19, 20].

2.) In page 6, please provide a reference of human plasma-DDP concentration and mention the time after treatment or other conditions (if any).

Answer: Thanks for mentioning this. We have now mentioned it and proved the reference.

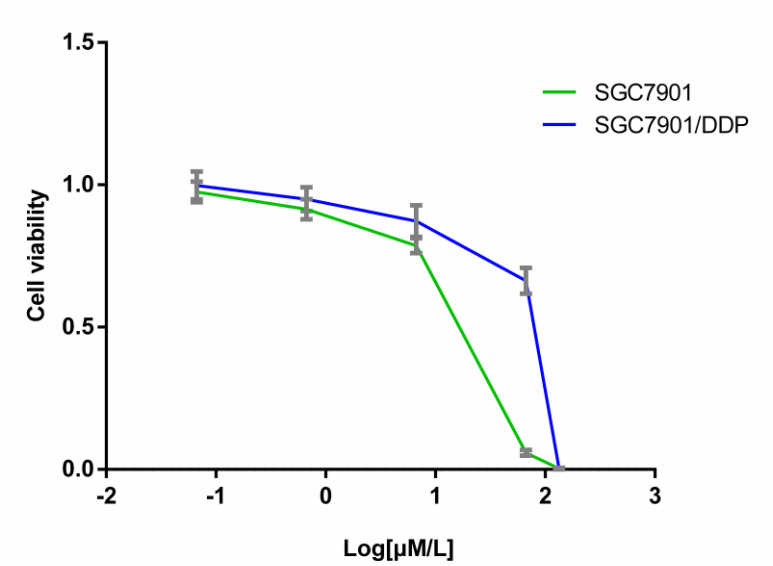
because the human peak plasma concentration for DDP has been reported as 6.67μM/L[21].

3.) In methodology section the authors said that they used the Agilent LncRNA-mRNA Human Gene Expression Microarray V4.0., but there are no information related to non-coding RNA genes and their presence in resistance related pathway analysis.
Answer: Now is not convenient to reveal information about LncRNA, because of this closely related our studying experiments.

4.) In methodology and result section please provide the logical explanation of bioinformatics study design (eg. Uses of KOBAS associated 7 pathways and 5 disease database), procedure and outcome information and its clinical interference. Please present your data more understandable form.
Answer: These previous studies were conducted in long non-coding RNA and mRNA bioinformatics study, while our studies were conducted in mRNAs analysis. We have now improved our interpretation and cited this reference into our methods section.
The entire analysis process includes two steps: first, bring the input gene ID map to the gene in the databases, and then annotate pathways, disease and function of these genes involved in. Second step, compare the first step results with background (usually the entire genome of the gene, or all the probe on the chip), unearth statistically significant enrichment pathways, disease or function. Fisher's exact test and $\chi 2$ test were used as statistical tests and the FDR was performed to correct the P-value [22]. Additionally, we used STRING 9.1 software to decipher the protein-protein interaction (PPI) network of the differentially expressed proteins. The PPI network may help in understanding the molecular mechanism of cisplatin resistance.

5.) All Figures and figure-legends should be presented properly in understandable form.
Answer: We greatly appreciate the reviewer for pointing this out. Please see answer to question 6-10.

6.) Figure 1: two cell line data can be compared in the same graph.
Answer: We have followed the reviewers' recommendation and compare the two cell line data in the same graph.



7.) Figure 2: Figure labellings is not clear, label the experimental groups in readable form and if possible mention the statistical procedure (in brief) in the legends.

Answer: We have improved explanation for the figure labelling as follows. Statistical procedure show up in the materials and methods section(Page 6).

Fig. 2. The mRNA expression levels from microarray. A: The volcano plot image showed the mRNA expression levels of microarray in SGC7901/DDP cells compared with SGC7901 cells. Black dots: equally expressed mRNAs between SGC7901/DDP cells and SGC7901 cells (FC ≤ 2); red dots: mRNAs were over-expressed in SGC7901/DDP cells compared with SGC7901 cells (FC ≥ 2); green dots: mRNAs in SGC7901/DDP cells were down-expressed compared to SGC7901 cells (p-values < 0.05, FC ≥ 2). Fold changes of these mRNAs in SGC7901/DDP cells compared with SGC7901 cells are shown as mean ± standard deviations. B: Two-dimensional hierarchical clustering image of the 1308 dysregulated mRNAs in the SGC7901/DDP cells compared with the SGC7901 cells, each row represents an mRNA, each column represents a sample. 7901-1, 7901-2 and 7901-3 represent the three samples of SGC7901 cells, DDP-1, DDP-2 and DDP-3 represent the three samples of SGC7901/DDP cells. Red: higher expression levels, green: lower expression levels.

8.) Figure 4: same as figure 2, labellings are not clear, label it in readable form and if possible mention the statistical procedure (in brief) in the legends.

Answer: The improved figure labelling as follows. Statistical procedure show up in the materials and methods section (Page 6, reference 22).

Fig. 4. Bioinformatic analysis of differentially expressed mRNAs. Gene ontology analysis of mRNAs dysregulated in SGC7901/DDP cells compared with SGC7901

cells. A: Top 30 molecular functions of the dysregulated mRNAs may associated with. Gene ontology analysis include biological processes, cellular components and molecular function. B: Gene ontology enriched diseases .Top 30 diseases annotations of dysregulated mRNAs may involved in. The disease enrich system include 5 disease databases: OMIM, KEGG disease, FunDO, GAD and NHGRI GWAS Catalog.

9.) Figure 5: Required more information and explanation in the legend to understand the experiment.

Answer: More information and explanation as follows.

Fig. 5. Heat-map of GO enriched cisplatin resistance pathways and input mRNAs which significantly altered in SGC7901/DDP cells compared with SGC7901 cells. a: PI3K-Akt signaling pathway and input genes b: MAPK signaling pathway and input genes c：Notch signaling pathway and input genes d：ErbB signaling pathway and input genes e: Jak-STAT signaling pathway and input genes f: NF-kappa B signaling pathway and input genes g：HIF-1 signaling pathway and input genes h：MicroRNAs in cancer and input genes. Each row represents an mRNA , and each column represents a sample. The intensity of the color indicates the relative levels of mRNAs. Red: higher expression levels, green: lower expression levels.The name of the input mRNAs which significantly altered (p-values < 0.05, FC ≥ 2) is present at the right of the figure.

10.) Figure 6: this figure is totally unclear not in readable form. Thickness of lines and others are very hard to understand in the interaction map.

Answer: We have done our best to improve the image clarity, but it contains so many proteins and protein-protein interactions, which just draw little useful information for us, this is also the only fly in the ointment.

11.) There are some typo mistakes, “p” should be replaced with p-values.

Answer: We have now corrected that.