Author's response to reviews

Title: ERCC1 and BRCA1 mRNA expression levels in metastatic malignant effusions is associated with chemosensitivity to cisplatin and/or docetaxel

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Author's response to reviews: see over
Reviewer 1
★ 1) p5-6: I have several questions ……

Reply on question of culture medium: Being widely used in ATP-TCA assay, the Completely Assay Medium (CAM) was adopted in 6-days cells cultures in our anti-cancer drug sensitivity assays. CAM culture system is designed to support the growth of tumor cells by endogenous cell factors and to limit the survival of normal cells in 6-days culture [1]. According to the previous reports, the serum free medium and polypropylene 96-well “U” microplates can enrich tumor cells up to 80-90%, reducing potential interference of non-tumor cell [1, 2]. And we have also proved the tumor cells number and viability by the end of 6-days culture in our preliminary test.

Reply on question of IC 50: Several parameters, such as Index SUM [3, 4], IC 50 and IC 90 [2] are often used to analysis the chemosensitivity to certain drug in ATP-TCA assay. Previous studies have shown that the Index SUM to be superior to the IC50 for determination of sensitivity and resistance as this relates more closely to the shape of the concentration-inhibition curve [5, 6]. So we used Index SUM to compare sensitivity or resistance to docetaxel in this study.

Reply on question of MI: a positive control containing maximum ATP inhibitor instead of docetaxel or cisplatin.

★ 2) Fig 3b: Most of the correlation seems to be due to the two extreme points. What happens if these points are eliminated?

Reply: After the two extreme points were eliminated, the significance was not found between BRCA1 mRNA expression and docetaxel chemosensitivity in gastric cancer group. However, firstly, we have checked again on these two points and verified the corresponding BRCA1 mRNA expression level. Secondly, in the analysis, we have noticed the potential effects that the outliers might have on the correlation coefficient. So a bootstrap method was used to examine the correlation and the result showed that the correlation coefficient was significant (P=0.019). Taking these into consideration, we kept the points in the data.
3) The authors should include another figure …..

**Reply:** In the present study, ANOVA instead of correlation assay was used to evaluate the interaction of ERCC1 and BRCA1 on sensitivity to cisplatin. I am sorry for that I do not know how to draw that figure to show this kind of interaction effect, but if it is necessary, I will try my best to do it 😊

**Reference**


**Reviewer 2**

★ The main question from the reviewer and question No. 1 and 9 are all caused by the pre-chemotherapy patients involving in this study, so we would like to explain together on this issue.

**Reply:** Forty-six patients who have been histological or cytological diagnosis as stage IV malignant disease were included in our study. Among them, only nine gastric cancer patients have received adjuvant or palliative chemotherapy before, and after a period of time, they occurred malignant ascites because disease recurrence or progress. That means, chemotherapy preceded the appearance of malignant effusions. So, the pre-chemotherapy of these patients should not have much effect on the in vitro chemosensitivity test.

We have made it more clearly in the revised manuscript, and many thanks to the reviewer for his or her valuable suggestion.
2. Page 3, line 19: chemositivitiy is misspelled. That occurs at different places in the manuscript.

**Reply:** We have corrected them and felt very sorry for it.

3. Page 4: in the last alinea it is stated that all malignant cells are metastatic. How did they know that they were not the primary tumors? How was the distribution of cancer cells in the malignant fluid? It is stated that the fluid contained at least 50% of cancer cells.

**Reply:** Metastasis from a lung, gastric or gynecological tumor is quite common etiology for the presence of malignant cells in effusions. In the present study, all patients were diagnosed as NSCLC, gastric or gynecological cancer, with malignant effusions at the time of diagnosis or disease progression. Furthermore, we exclude the malignant mesothelioma (MM), the common reason of primary malignant pleural or peritoneal effusions by patients’ histological, imaging as well as pathological characteristics of tumor cells in effusions.

Cancer cell is floated in the effusions. After centrifugation, smears of cell pellets underwent morphologic evaluation by pathologists in our hospital. The specimens comprising at least 50% cancer cells were included in this study. This was a main reason for our relatively small samples in this study as we have screening nearly 70 patients with malignant effusions, among them, only 46 patients’ samples qualified the above criteria.

4. Page 5, several grammar errors. line 4: the interface was collected. This should be clarified.

**Reply:** We have modified it as “the interface layer was collected”.

5. Page 5, 3th alinea: appropriate positive and negative controls. Which controls?

**Reply:** Commercial RNA and RNase-free water were used as positive and negative control separately. We have made it clear in revised manuscript.

6. Page 5, last alinea: explain why these test drug concentrations are used.

**Reply:** Those concentrations were six doubling dilution of test drug concentrations
(6.25% to 200% TDC). TDC values were determined by pharmacokinetic and clinical information and were widely used in ATP-TCA method. In the present study, TDC values of cisplatin and docetaxel were determined according to the previously reports (Andreotti PE, et al. Cancer Res 1995, 55: 5276-82 and Tian HM, et al. Oncol Prog 2005; 3: 436-441).

7. Page 7: the figure numbers are not corresponding with the figures in the text. The language is confusing. Was ERCC1 expression not related to docetaxel sensitivity?

**Reply:** According to the previously reports, ERCC1 is closely related with cisplatin however BRCA1 is related with both cisplatin and taxane sensitivity in some kind of tumor (the details have been described in the introduction part of manuscript). The aim of this study is to investigate the relationship between ERCC1 and cisplatin, BRCA1 and cisplatin as well as docetaxel in malignant effusions. So we did not discuss the relation between ERCC1 mRNA expression level and docetaxel sensitivity in the manuscript.

8. Discussion: Second alinea: Response to chemotherapy does not depend on intrinsic chemosensitivity alone. Discus the other factors and the impact of those factors in relation to the in vitro method they used.

**Reply:** Response to chemotherapy does not depend on intrinsic chemosensitivity alone. A patient’s chemoresponse depends on other factors as well, including drug metabolism *in vivo*, blood supply to the tumor, patients’ fitness to undergo chemotherapy and so on. However, previous studies with the ATP-TCA suggest that the assay is a good model for the investigation of tumor chemosensitivity and the results so far show good correlation with clinical trial results. In addition, as patients usually will be treated by combination chemotherapy in clinic, the adopting of in vitro assay in the present study made it possible to avoid interference from other drugs.

10. Table 1: mention the types of NSCLC.

**Reply:** The pathology type of NSCLC in this manuscript is adenocarcinoma.
11. Make the Y-axis of the different figures the same so that comparison is easier to make. It is not clear for the reader what the relative ERCC1 expression in figure means. That should be explained. Moreover, in the text on page 6 a range for ERCC1 expression is mentioned of 0.005-0.831.

Reply: We have modified figure and figure legends according to reviewer’s suggestion in the revised manuscript. The range of relative ERCC1 expression is does 0.005-0.831. When the correlation between ERCC1 mRNA expression and drug sensitivity was analyzed using spearman correlation method, the value of ERCC1 expression was logarithmically transformed, as appeared in Y-axis of the figure.