Author's response to reviews

Title: Association of decreased mitochondrial DNA content with colorectal cancer progression

Authors:

haihong cui (hh_cui@sina.com)
ping huang (hp_20050909@126.com)
ying han (hanying@sina.com)
yunxin zhang (zhangyx@sina.com)
zhennhua zhang (zhangzh@sina.com)
wei xu (18910998563@189.cn)
xiaopeng wang (wangxp@126.com)

Version: 4 Date: 7 January 2013

Author's response to reviews: see over
Research article

Association of decreased mitochondrial DNA content with colorectal cancer progression

haihong cui, ping huang, ying han, yunxin zhang, zhenhua zhang, wei xu and xiaopeng wang

BMC Cancer (Section: Genetics, genomics and epigenetics)

Thank you for consideration of our manuscript for publication in your journal. We have reviewed the above manuscript according to your reviewer’s comments.

Reviewer#1 (Dr Lee):
The authors analyzed mtDNA copy number from 60 patients with colorectal cancer and showed a correlation between a reduced copy number of mtDNA in colorectal cancer and lymph node metastasis. The small sample size might limit the significance of this study. The authors should point out the potential limitations in this study. In addition, a lot of drawbacks are found in the manuscript.

- At the last sentence of our article, we have pointed out the potential limitation in our study.

1. The authors should provide clear methods and detail experimental conditions, such as the real-time quantitative PCR analysis (Cyber green? What kind of polymerase was used?). It should be clearly quantified for the template DNA for standard curves, including nDNA (#-actin) and mtDNA (ND1). Were they obtained from the cloned DNA fragments inserted in a plasmid?

- We have described the method of real-time PCR in detail, including sybergreen-I polymerase, The PCR conditions and a standard curve was presented by recombinant plasmids serially diluted 10^4 to 10^8 times.

2. It is not clear whether any criteria were used for selecting these 60 patients. If any, they should be stated in the Materials and Methods section

- In the Materials and Methods section, we have added detailed recruit criteria.
3. In the Discussion section, several statements lack citations. Many statements are confused for readers including the sentences on Ref 16 and Ref 17.

- We have deleted the cell line in the sentences on Ref 16, and revised the sentences that a source of confusion on Ref 17.

4. The authors should make some discussions about many other papers that have been published regarding mtDNA content associated with cancer progression, including gastric cancer, breast cancer and renal cell carcinoma.

- At the first paragraph of Discussion, we have added some reference of cancer regarding mtDNA content.
Reviewer#2(Dr Tseng):
This manuscript analyzed mtDNA copy number from 60 patients with colorectal cancer and showed a correlation between a reduced copy number of mtDNA in colorectal cancer and lymph node metastasis. No other significant correlation was found. There are several drawbacks in the manuscript.
1. There are a lot of typo errors in all text. English edition is needed.
   • Done.

2. In the Materials and Methods section, the authors should provide clear methods and detail experimental conditions, such as the real-time quantitative PCR analysis (cyber green or TaqMan? What kind of polymerase was used?). It is not clear what templates were used for obtaining standard curves.
   • We have described the method of real-time PCR in detail, including sybergreen-I polymerase.

3. For the 60 patients, it is not clear whether any criteria were used for selecting these patients. Are all specimens obtained without any treatment?
   • We have added the selected criteria in materials and methods.

4. In the Discussion section, several statements lack citations.
   • We have done this question.

5. In the discussion for reference 16, it is not a study on hepatocellular carcinoma cell line.
   • We have revised this sentence.

6. In the discussion for reference 17, it is not the authors’ finding, but they stated “we can detect…”?
   • We have modified the sentence.

7. Many papers have been published regarding mtDNA content associated with cancer progression, including gastric cancer, breast cancer and renal cell carcinoma. The authors should make some discussions.
   • We have added other references about the issue.

8. This study analyzed a small size (60 cases) of samples. The small sample size might limit the significance of this study. The authors should point out the
potential limitations in this study.

• At the last paragraph, we have explained the potential limitations in our study.