Author's response to reviews

Title: Overexpression of UbcH10 alternates the cell cycle profile and accelerate the tumor proliferation in colon cancer

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Response to the comments of the Editor and Reviewers

January 2, 2009

Dear Dr. J. A. Le Good:
Senior Assistant Editor
BMC-series journals

We thank you and the reviewers for reviewing our manuscript entitled “Overexpression of UbcH10 alternates the cell cycle profile and accelerate the tumor proliferation in colon cancer” [pre-revised title: Functional analysis and clinicopathological relevance of UbcH10 in colon cancer.”] The comments were very constructive and helped in enhancement of our manuscript. We have assessed the potential role of UbcH10 in tumor suppression by a combinatorial approach of human tissue array and cell biology analyses. In particular, UbcH10 functions as an E2 ligase involved in the spindle assembly checkpoint and thereby averts abnormal progression into M phase. Our result demonstrates a pathological correlation between UbcH10 and colon cancer progression and reveals a potential novel biomarker for colon cancer. We believe that our study provides important insight into the mitotic regulation of UbcH10 and links UbcH10 with colorectal malignancies, and will be of interest to an audience from the field of ubiquitin/cell cycle and tumorigenesis.

We are pleased to report that we have addressed to most of the reviewers’ specific comments and revised the manuscript accordingly.

Importantly, however it is true that there is limitation of time (we have received the reviewers comments on December 13 2008 and suggested deadline is January 2 2009). Therefore, we were afraid that we could not fully accomplish the revision.

Thank you for your consideration of our manuscript for publication.

Sincerely yours,

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Response to Reviewer 1

We appreciate the constructive comments by Reviewer 1. We hope that the reviewer will find our responses satisfactory.

**Major comments**

1. Reviewer 1 suggested to incorporate the recent important two publications. We incorporate these articles into our manuscript.

2. Reviewer 1 suggested to consider the recent articles discussing about the significance of UbcH10 in various type of cancers other than gastrointestinal malignancies. This is very constructive comment, and we incorporate suggested articles into our manuscript.

3. Reviewer 1 requested to provide the detailed clinical information for the patients with colon cancer. We could provide the major clinical data of the colon cancer patients (age, gender, TNM status, and histological grade of the tumor) in Table 1. However due to the limitation of the information, we just provide these clinicopathological information in this manuscript.

4. Based on the interesting suggestion from reviewer 1, we have immunohistochemically tested the status of Ki-67 in colon cancer tissues and found that there were significant correlation between the level of UbcH10 and Ki67. This result is compatible with recent demonstration. Currently Lei, et al demonstrated the increased UbcH10 labeling index in high-grade astrocytomas compared with low-grade tumors or normal control, and moreover a positive correlation between UbcH10 immunoreactivity and Ki-67 immunostaining was also noted. Therefore, these observations suggest that UbcH10 is potentially important indicator to predict the aggressive behavior of certain type of tumors.

5. Reviewer 1 concerned about the possible association between the status of UbcH10 and patients survival. However due to the limitation of the information, particularly the patients survival (moreover, these patients were composed of groups with different adjuvant treatment strategy), we consider that it is not suitable to provide survival information of these patient. Additionally, histological grade is well known to be correlate with the aggressive behavior of the tumor. Therefore, it is possible that result of this study could indirectory compensate to reflect the patients survival information in which patients with higher level of UbcH10 could suffer more aggressive disease.

**Minor comments**

We collected the several spelling errors in the manuscript.
Response to Reviewer 2

Comments from Reviewer 2 were very constructive summarizing that “The data even though only partially novel are interesting confirming the potential role of UbcH10 detection as a novel marker of cancer progression.”
In reviewer2’s comment, because ‘clinicopathological relevance’ could precede the functional studies in this manuscript, reviewer 2 raised a proposal for the different reorganization of the manuscript. The reviewer’s suggestion may be a little bit different from our concern about the flow of the presentation in our manuscript. However, he provided us the potential important point. Indeed, we did demonstrate that alternation of UbcH10 affect the cellular proliferation rather than the detail its biological mechanism. Therefore, the words ‘Functional study’ might not be the suitable to adequately describe our study. Due to the above issue, in this manuscript, we changed the title to the “Overexpression of UbcH10 alternate the cell cycle profile and accelerate the tumor proliferation in colon cancer”.

Response to Reviewer 3

Comments from reviewer 3 are constructive to strengthen our manuscript. He also concerned the phenomenon that overexpression of UbcH10 increased cellular proliferation despite the reduction of mitotic population. The following are our responses to the specific comments:

Major points
It has been widely recognized that increased number of mitotic cells would reflect the high cellular proliferation rate and aggressive behavior of tumor. However in this study and previous reports demonstrated that overexpression of UbcH10 accelerate the tumor growth while decreased the population in mitosis. As the reviewer 3 pointed out, this phenomenon is kind of contradictory to the traditional concept. This issue may be associated with the recent argument about the functional role of UbcH10 in cell cycle control. On one side, UbcH10 is reported to be important in both the G1/S transition and the initiation of metaphase to anaphase transition. However, on the other hand, the role of UbcH10 is currently suggested to be only at the end of G1 phase, being inconsistent with both in the spindle checkpoint and in inactivating the APC/C.
At this point, we are not able to fully address the underlie physiological function of UbcH10. However, based on these observations, two contradictory findings such as high cellular proliferation rate and low mitotic population, which induced by the overexpression of UbcH10 may be considered independently.
Reviewer3 also concerned about the possible association between the status of UbcH10 and patients survival. However due to the limitation of the information, particularly the patients survival (moreover, these patients were composed of groups with different adjuvant treatment strategy), we consider that it is not suitable to provide these patient survival information. Additionally, histological grade is well known to be correlate with
the aggressive behavior of the tumor. Therefore, it is possible that result of this study could indirectly compensate to reflect the patients survival information in which patients with higher level of UbcH10 could suffer more aggressive disease.

Minor points
1. We collected the misspelling words as “late mitosis” in page 3 line 16.
2. We revised the sentence as “69 patients (46%) were histologically positive for UbcH10” in page 9.
3. We collected the misspelling in page 9 as “there were no significant differences”. Likewise, we collected the misspelling in the legend to Figure 4 “…in patients …”.
4. We added the literature discussing about the overexpression of UbcH10 in breast cancer in the references.
5. We collected the misspelling in the legend to Figure 1C as “normal colon epithelia”.
6. We do not agree with the reviewer’s suggestion, we did not remove the labels A and B in Figure 1D.
7. We did establish the two stable clones over-express the UbcH10, and cellular behaviors were similar between these clones compared with the control. Therefore, in this study, we presented the representative data obtained in one of the clones.
8. The populations of the cells in G2/M were significantly different both in Figure 2D, and 3D.

Response to Reviewer 4

Reviewer 4’s comments were positive summarizing “The pictures are really convincing and the paragraph also, … If this is added, this part of the article will become of great interest”. Reviewer 4 raised a couple important questions concerning the manuscript. The following are responses to the specific comments:

Major comments
1. The reviewer 4 concerned the important point, which could explain and unveil the potential association of UbcH10 and cancer. In our previous reports, we demonstrated that over-expression of UbcH10 in cancer cells promote the mitotic exit resulted in the impairment of proper mitotic progression. Based on these observations, we are exploring whether aberrant level of UbcH10 would affect the status of chromatid using the spectrokaryotype, then investigating the potential link between UbcH10 and initiation or
progression of cancer as the ongoing project. We hope we can you the results in our future manuscript.

2. It has been widely recognized that increased number of mitotic cells would reflect the high cellular proliferation rate and aggressive behavior of tumor. However in this study and previous reports demonstrated that overexpression of UbcH10 accelerate the tumor growth while decreased the population in mitosis. As the reviewer 4 pointed out, this phenomenon is kind of contradictory to the traditional concept. This may be associated with the recent argument about the functional role of UbcH10 in cell cycle control. On one side, UbcH10 is reported to be important in both the G1/S transition and the initiation of metaphase to anaphase transition. However, on the other hand, the role of UbcH10 is currently suggested to be only at the end of G1 phase, being inconsistent with both in the spindle checkpoint and in inactivating the APC/C.

At this point, we are not able to fully address the underlie mechanism. However, based on these observations, two contradictory findings such as high cellular proliferation rate and low mitotic population, which observed in the over-expression of UbcH10 can be discussed independently.

3. There are remarkable changes of doubling times by the alteration of UbcH10. Calculated doubling times of DLD1 cells; 37.03 hr (control cells), 21.48 hr (UbcH10-overexpressed cells), and 53.69 hr (UbcH10-depleted cells), respectively.

4. We do have the quite similar results obtained by the alteration of UbcH10 in breast cancer cells (Fujita, et al. Cancer Sci 2008), where over-expression of UbcH10 resulted in the increase in cellular proliferation and reduced the population on mitosis. However, we have never tested using the cells with different p53 and K-ras status with the suitable control.

5. We revised the Figure 4C following the suggestion from reviewer 4

Minor comments

1. Reviewer 4 concerned whether the alteration of the S-phase population would be occurred in UbcH10-overexpressed cells, and this point is quite noteworthy. Because originally the functional role of UbcH10 is mainly understood to regulate the mitotic exit, therefore we particularly focused on the mitotic progression and tried to link the cellular proliferation in this study. However it is also true that overexpression of UbcH10 increased the S-phase population, and this phenomenon was confirmed both in our examinations (detail data was not shown) and previous investigations. Recent study suggested that UbcH10 has also important role at G1/S phase, where Cyclin A can accumulate in late G1 only after UbcH10 induced its own ubiquitination and degradation. Therefore, we add these discussions in our manuscript.

2. Reviewer 4 concerned that whether alteration of UbcH10 affects the level of Usp44. We totally agree with his interesting implication, and actually we begun to investigate the
potential involvement of Usp44 in cancer in our ongoing project. We hope that we can address the concern raised by reviewer 4 in future manuscript.

3. Since we have not tested the level of UbcH10 under the treatment with UbcH10, we could not address the question. However since it was previously demonstrated that level of UbcH10 is controlled by its own ubiquitination and degradation, it is quite reasonable that proteasome inhibitors may act as the inhibition of UbcH10 expression.

4. We revised the Figure 1B with error bar in manuscript.

5. We agree with the opinion of reviewer 4 describing that it is unknown whether UbcH10 is ‘cause’ or ‘consequence’ of cancer. Following the suggestion raised by reviewer 4, we revised the description of manuscript.

6. We collected the misspelling pointed by reviewer 4