Author's response to reviews

Title: Argonaute proteins: potential biomarkers for human colon cancer

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Author's response to reviews: see over
Dear editors,

Thank you for your letter on November 25 concerning our manuscript “Argonaute proteins: potential biomarkers for human colon cancer” (Manuscript number: 5451233982679361), together with the comments from reviewers.

We have revised the manuscript in accordance with the reviewers’ comments. We are returning here the revised manuscript with a point-by point response to the concerns of reviewers and a description of the changes made.

We would like to thank the referees for their useful comments and hope that the revised manuscript is acceptable for publication in BMC Cancer.

Best Regard,

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Response to the concerns of Reviewer 2

Comment: The authors should attempt to validate to what extent the Agos or PIWI are involved in cancer progression. One way to do this would be to suppress the respective Agos or PIWIs in colon cancer cell lines and determine what effect this has on the colon cancer cells division, metastasis, or overall fidelity. One could easily knockout the Agos or PIWIs using U1 adapters (Goraczniak, Behlke et al. 2009) or antisense ODNs. Such experiments would assist in supporting the notion that the Agos or PIWIs are somewhat functional in cancer cell fidelity.

Respond: The reviewer’s comments are very helpful and important. We understand that to determine the effect of Agronaute proteins by down-regulation of Agos or PIWIs expression may better validate to what extent the Agronaute proteins are involved in cancer progression. However, in the present study, our main aim is to investigate the expression of human Argonaute family in colon cancer and the correlation of protein expression profiles with patients’ clinicopathological features which has not been studied before, and we think that what we have done may not be optimal, but should be sufficient to draw a conclusion that Argonaute proteins might be protential biomarkers for human colon cancer.

In addition, several previous studies (Lee et al., 2006; Liu et al., 2006) have reported that the gene silencing of PIWIs by RNAi or antisense technology inhibited the growth of cancer cells and induced apoptosis in human gastric cancer and seminomas, which supported the issue that Agronaute proteins were somewhat functional in cancer development and progression. In the revised paper, we have cited these studies as references in the discussion section (page 11, paragraph 2). Together with these observations, we believe the conclusions from our study are reliable.

It will be of great interest to initiate some research on the function of Agronaute proteins in colon cancer. Thank you for the sincere comments about this issue and further studies are underway to determine the effect of Agronaute proteins in colon cancer by direct down-regulation of Agos or PIWIs expression using antisense ODNs or RNAi techniques.
Response to the concerns of Reviewer 4

Comment:
The authors considered most of my comments in their revised manuscript. However, I still do not understand what they want to say with table 5 and the statement: Logistic regression analysis revealed that the positive expression of EIF2C1 and PIWIL2 was associated with a significantly increased risk of colon cancer. All patients were colon cancer patients. What does the parameter risk of colon cancer mean? Do they compare expression levels in tumor tissue and in normal tissue? Do they want to say positive expression of EIF2C1 and PIWIL2 was correlated with an increased risk of tumor-related death for colon cancer patients? If yes, how did they calculate this? This comment refers to previous comments 2, 4 and 6.

Respond: The reviewer’s comments are very helpful and important. Some sentences were incorrectly stated in our revised manuscript and cover letter. The explanation is stated as following:

Data from 150 tissue samples, including 75 tumorous specimens and 75 adjacent non-tumorous specimens were used for logistic regression analysis. The independent variables were the expression levels of EIF2C1-4 and PIWIL1-4, and the dependent variable, histotype of colonic lesion, had a value 1 if the colonic lesion is tumorous tissue and 0 if the colonic lesion is adjacent non-tumorous tissue. Logistic regression analysis revealed that the colonic lesion with positive expression of EIF2C1 (OR = 3.071, \( P = 0.005 \)) and PIWIL2 (OR = 7.392, \( P < 0.001 \)) was associated with a significantly increased risk of tumorous tissue (Data are shown in Table 5). However, the rest of human Argonaute proteins had no significant effects on histotype of colonic lesion.

According to this explanation, the relevant changes have been made in the Table 5 as well as in the “Result” section and “Discussion” section (page 8, paragraph 3 and page 11, paragraph 2). We are grateful to the reviewer for pointing out our error.

Previous comment 4:
Several sentences need urgently to be revised:
Abstract: Furthermore, Logistic regression analysis revealed that the risk factors of
colon cancer were the positive expression of EIF2C1 and PIWIL2. There can be a
correlation but an expression can not be absolutely the risk factor itself.

Respond: Page 2, paragraph 3: The sentence has been revised to “Logistic regression
analysis revealed that the colonic lesion with positive expression of EIF2C1 and
PIWIL2 was associated with a significantly increased risk of tumorous tissue”.

Previous comment 6:
Several sentences need urgently to be revised:
Discussion: ... we observed that positive expression of EIF2C1 and PIWIL2 were
independent predictors of colon cancer. A protein expression can not be the predictor
of a cancer type!

Respond: Page 11, paragraph 2: The sentence has been revised to “we observed that
colic lesions with positive expression of EIF2C1 and PIWIL2 were at increased risk
of tumorous tissue.”

Reference

Lee JH, Schütte D, Wulf G, Füzesi L, Radzun HJ, Schweyer S, Engel W, Nayernia K:
Stem-cell protein Piwil2 is widely expressed in tumors and inhibits apoptosis

Expression of hiwi gene in human gastric cancer was associated with