Author's response to reviews

Title: Reduced expression of p21-activated protein kinase 1 correlates with poor histological differentiation in pancreatic cancer

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Version: 3
Date: 5 June 2014

Author's response to reviews: see over
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Title: Reduced expression of p21-activated protein kinase 1 correlates with poor histological differentiation in pancreatic cancer

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Version: 2 Date: 5th June 2014

Author's response to reviews: see over
The Biomed Central Editorial Team

**Object:** MS: 9694234061241774 - Reduced expression of p21-activated protein kinase 1 correlates with poor histological differentiation in pancreatic cancer. *Dr Juan Han et al.*

Thank you for consideration of our manuscript for publication in BMC Cancer. We have revised the above manuscript according to the reviewers' comments.

**Reviewer # 1(Dr Hong He)**

Concerns:

1. In table 2, the percentages calculated should be low or high PAK1 expression cases vs total (low+none+high) cases determined per category, e.g. ages the percentage of high PAK1 expression in patients # 60 should be calculated as 26/(15+26)=63.41%; likewise, the percentage of high PAK1 expression in patients> 60 should be 12/(19+12)=38.71%; thus the p value should be calculated as 63.41% vs 38.71% rather than stated in page 10 line 169, 68.4% vs 31.6%.

   We have revised table 3 as the reviewer recommended.

2. How statistically significant is the rate of high PAK1 expression in well differentiated samples since there are only 3 cases? How is the rate (64%) of high PAK1 expression in clinical stage IV compared with the rate of high PAK1 expression in clinical stage I-III?

   Well-differentiated tumor accounts for a small portion of pancreatic cancer. As a study shows, only 4.5% of all pancreatic cancer are well-differentiated (Surgery. 2012;152:S112-9). We observed a similar...
rate of well-differentiated tumor in our study (3/72 = 4.2%). As the reviewer indicates, it is hard to get a conclusion with only 3 cases in well differentiated group. A study from SEER database showed survival was significantly worse for high-grade (poorly/undifferentiated) versus low-grade (well/moderately differentiated) tumors (Ann Surg Oncol. 2010;17:2312-20). Therefore, we combined the moderately-differentiated and well-differentiated samples together and analyzed the data again. As table 3 shows, the rates of high PAK1 expression in poorly- and moderately/well differentiated samples were 39.0% (16/41) and 71.0% (22/31) respectively. PAK1 was still associated with pathologic differentiation (p= 0.007). We have revised the texts on page 10, line 169 to line 170 and table 3 accordingly.

1 Stage IV is advanced stage composed of patients with metastasis. The prognosis of stage IV patients is significantly poorer than stage I-III patients. Therefore, we divided patients into stage I-III and stage IV in analysis.

3. In the category of “Pathologic Differentiation”, the authors demonstrated that poor pathological differentiation was associated with low PAK1 expression while significant high PAK1 expression was found in moderate and well differentiated samples. How is this significance in PAK1 expression in term of pathological differentiation reflected in the PAK1 expression in term of clinical stage?

1 As the reviewer pointed out, PAK1 was associated with pathologic differentiation (p= 0.007). Moderately/well differentiated tumors have significantly higher PAK1 expression than poorly differentiated tumors (71% vs 39%). But there was no significant correlation between PAK1 protein expression and clinical stage. Stage I-III tumors have similar PAK1 high expression as stage IV (50% vs 64.3%, p=0.706).
4. In the Discussion part, page 13, line 231, the authors asserted that “The expression of PAK1 in human pancreatic cancer has never been reported”, which is not so. Jagadeeshan et al., reported that PAK1 expression was deregulated in pancreatic ductal adenocarcinoma (PDAC) from patients (Oncogene 2014) and pancreatic cancer cells. Both Jagadeeshan et al., and Yeo et al., (Cancer Letters 2014) reported that PAK1 promoted pancreatic cancer cells growth in vitro and in vivo. The authors should take considerations of these reports when discussing their results.

   As the reviewer suggested, we have deleted the sentence “The expression of PAK1 in human pancreatic cancer has never been reported” in page 13.
   We have added the discussion about the recent two papers (Jagadeeshan et al, Oncogene 2014, and Yeo et al., Cancer Letters 2014) in page 13, line 233 to 238 and line 243 to 244.

5. Since the survival data were obtained from primary pancreatic cancer patients, and there was no clear the data presented in the paper showing the role of PAK1 expression in the liver metastatic pancreatic cancer, therefore the title “High expression of P21-activated protein kinase 1 is an independent prognostic factor in primary and metastatic pancreatic cancer” should be changed to exclude “metastatic” to present the proper information provided in the paper.

   As the reviewers recommended, we have changed the title “High expression of P21-activated protein kinase 1 is an independent prognostic factor in primary and metastatic pancreatic cancer” to “Reduced expression of p21-activated protein kinase 1 correlates with poor histological differentiation in pancreatic cancer.”
Reviewer # 2 (Dr Takeo Shimasaki)

Major comments:

1. This paper shows good experimental work, but statistical analysis is inadequate. The method and results of multivariate analysis in relation to a confounder are not described well enough. Because the expression of PAK1 correlates with a histological type, the exclusion of the confounder is an important point to consider during validation of an independent prognostic factor. The situation is similar with clinical staging. If the authors insist on it, they have to at least prove that PAK1 is an independent factor regardless of a clinical stage and the degree of differentiation. (Please see specific comment 1)

1. A statistician Dr Ying Guo was invited to do the statistical analysis. She is added as a co-author. Multivariate Cox regression using enter, forward and backward methods was utilized to analyze the effect of clinical stage, PAK1, histological grade, radiotherapy, chemotherapy, age and gender in primary pancreatic cancer patients. Using multivariate analysis with different combinations of those variables for confounder factors adjustment, we have established a stable and robust analysis model. The results of multivariate analyses showed that clinical stage and PAK1 are prognostic factors for primary pancreatic cancer. In addition, interaction analysis also showed that there was no interaction effect between clinical stage and PAK1. So PAK1 is an prognostic factor for primary pancreatic cancer. Please see the answers to specific comment 3.

2. There are no data on the expression of PAK1 in the liver metastatic tissue and during pathological differentiation. Contrary to previous reports, the authors state that PAK1 expression is higher in primary pancreatic cancer
tissues than in metastatic tissues. In contrast, this manuscript lacks the data on the number of metastatic pancreatic cancer patients according to histopathological differentiation. Without the detailed data on metastatic pancreatic cancer, referees and readers could not understand the title.

1. We have added table 2 clinical and pathological characteristics of 20 patients with metastatic pancreatic cancer. As the reviewer suggested, we have changed the title to “Reduced expression of p21-activated protein kinase 1 correlates with poor histological differentiation in pancreatic cancer”.

3. This PAK1 expression in the metastatic tissue can be under the influence of differentiation and metastatic signs during diagnosis. I cannot understand and agree with the results without data on histopathological differentiation and clinical outcomes. In particular, to describe the relationship of metastasis with PAK1, the authors should describe the clinical definitive outcome of the metastasis. The samples and clinical data are usually collected and analyzed at the point of diagnosis. When cancer progression is observed after treatment, histological studies are generally not performed. I think that these data from histological evaluation, which are suggestive of metastatic tissue, should not be analyzed only in metastatic cases at an early stage of clinical course. It is not appropriate to search for correlations between PAK1 expression and the presence of metastases at diagnosis. Furthermore, we cannot rule out the possibility that the results will change if the metastatic cases are added to the middle and the end of the clinical course. If the authors would like to discuss the metastasis and PAK1, they should compare PAK1 expression in a primary pancreatic cancer with or without a definitive metastasis. As mentioned above, there are no data in this manuscript that supports the statement in the title.
We agreed with the reviewer that the results will possibly change if the metastatic cases are added to the middle and the end of the clinical course. While it’s extremely to get tumor samples after disease recurrence or at the end of the clinical course. So we don’t have the data to show that. The correlation between metastatic and non-metastatic pancreatic cancer tissues was analyzed in 72 primary pancreatic cancer patients, and there was no significance difference.

Specific comments:

1. The running title appears awkward in the present manuscript. One of the alternatives may be “Reduced expression of p21-activated protein kinase 1 correlates with poor histological differentiation in pancreatic cancer.” If the authors could demonstrate additional statistical data (i.e., a Kaplan–Mayer survival curve according to the degree of differentiation and clinical stages I, II, III, and IV and the results of statistical analysis), then I would agree with the phrase “an independent prognostic factor.”

As the reviewer suggested, we have changed the title to “Reduced expression of p21-activated protein kinase 1 correlates with poor histological differentiation in pancreatic cancer.”

2. Clinical and histopathological evaluation of metastatic cancer should be shown in a new table along with the number of cases, if the authors would like to include the data on metastatic pancreatic cancer.

As mentioned above, we have added table 2 clinical and pathological characteristics of 20 patients with metastatic pancreatic cancer.
3. The authors need to provide a detailed description of the statistical method of the multivariate analysis with adjustment factors.

Multivariate Cox regression using enter, forward and backward methods was utilized to analyze the effect of clinical stage, PAK1, histological grade, radiotherapy, chemotherapy, age and gender in primary pancreatic cancer patients. Using multivariate analysis with different combinations of those variables for confounder factors adjustment, we have established a stable and robust analysis model. The results of multivariate analyses showed that clinical stage and PAK1 are prognostic factors for primary pancreatic cancer.

Here are some examples of multivariate Cox regression analysis.

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<td>2(0.019), 3(0.007)</td>
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</tr>
<tr>
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<td>2(0.004),3(0.002)</td>
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<tr>
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<td>2(0.027),3(0.006)</td>
</tr>
<tr>
<td>1,2,4,5,6</td>
<td>None</td>
</tr>
<tr>
<td>1,3,4,5,6</td>
<td>3(0.037)</td>
</tr>
</tbody>
</table>

1,differentiation; 2,clinical stage; 3,PAK1 expression; 4, radiation; 5, chemotherapy; 6,age; 7,gender