

ANSWERING REVIEWERS



May 9, 2014

Dear Editor,

Title: PBX3 promotes migration and invasion of human colorectal cancer cells via activation of MAPK/ERK signaling pathway

Author: Haibo Han, Jin Gu, Dengbo Ji, Zhaowei Li, Yuan Zhang, Wei Zhao, Limin Wang, Zhiqian, Zhang

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 10302

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

Reviewer: 2908153

Questions:

1. The manuscript needs to be revised by a native speaker. For example: (1)first line of page 3: Colorectal cancer (CRC) is third most common type f cancer. (2)ninth line of page 3: have been reported to play an important role in tumor growth.
2. In the result part, we found Figure 1A was performed by reverse transcription-PCR. However, this experiment was not described.
- 3.Table 1, you should give us the range of the PBX3 expression. Tumor stage should be T1-T4.

Answers:

1. The manuscript has been revised by a native speaker, Arthur Berg PhD, associate professor of Biostatistics & Bioinformatics at Penn State College of Medicine Bioinformatics Core Director. This manuscript is also revised by Jing-Yun Ma Editorial Office: <http://majingyun.baikemy.com>. We have replace "f" into "of" and replace "have" into "has" on page 3.
2. Fill in the reverse transcription-PCR in Material and Methods, Result and Figure legend.
3. Fill in the range of the PBX3 expression. In our clinical data, only one patient belongs to T1 stage which is unable to calculate the significant difference by stastistic method, so we merge T1 and T2 stage together.

Reviewer: 77679

Questions:

General comments:

The issue is only one. The authors used only colorectal cancer cells. We want to know how about in other cancer cells.

Minor point:

- 1) Page 3, Line 2 from the bottom: Is mir-181 miR-181?
- 2) Page 4, Line 16: CO2 should be CO₂.
- 3) Page 4, Line 16: incubator is not necessary
- 4) Page 4, Line 19: are should be were.
- 5) Page 5, Line 9 from the bottom: (Invitrogen, Carlsbad, CA) should be (Invitrogen).

- 6) Page 6, Line 5: Add City name in Corning.
- 7) Page 6, Line 8: No need Wetzlar, Germany again.
- 8) Page 6, Line 15: 107 cells should be 10⁷ cells.
- 9) Page 6, Line 4: from the bottom: No need Valencia, CA again.
- 10) Page 7, Line 9: Add City and State name in Millipore.
- 11) Page 7, Line 9: Add City and State name in Eptomics.
- 12) Page 7, Line 12: Add City name in cwBiotech.
- 13) Page 7, Line 14: Remove USA.
- 14) Page 7, Line 5 from the bottom: t in t-test should be written by *Italic* style.
- 15) Page 10, Line 6 from the bottom: Fig. 2B should be Fig. 4B ?
- 16) Page 10, Line 4 from the bottom: phosphor-ERK1/2 should be phospho-ERK1/2.
- 17) Page 10, Line 3 from the bottom: phosphor-ERK1/2 should be phospho-ERK1/2.
- 18) Page 10, Line 1 from the bottom: Fig. 4B should be Fig. 4C ?
- 19) Page 16, Line 9 from the bottom: Expression of what? Q-PCR? If so, write that.
- 20) Fig 1A: show bp and kDa.
- 21) Fig 1A: The intensity of actin is not same. Please load the same amount of proteins samples.
- 22) Fig 2A: Lentivirus Control should be Lentivirus control.

Answers:

General comments: In this manuscript, we reported only colorectal cancer cells. In fact, we also detected the PBX3 expression in hepatocellular carcinoma specimens. Consistent with the result in colorectal cancer, the high expression of PBX3 in hepatocellular carcinoma tissues is associated with a bigger tumor size, early recurrence, low 4-years survival rate and also a shorter survival time. Furthermore multivariate COX regression analysis revealed that high level of PBX3 expression in cancerous tissues was an independent risk factor of bad prognosis for HCC patients. The unpublished data is submitted to another journal and hope it can be accepted. The high level of PBX3 expression was also reported to be associated with tumorigenicity and malignant property of human prostate cancer and gastric cancer by literature as shown in manuscript reference 10 and 14.

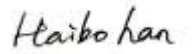
Minor point:

- 1) Replace "mir-181" into "miR-181".
- 2) Replace "CO2" into "CO₂".
- 3) Delete incubator
- 4) Replace "are" into "were".
- 5) Replace "(Invitrogen, Carlsbad, CA)" into "(Invitrogen) ".
- 6) Add City for Corning.
- 7) Delete Wetzlar, Germany.
- 8) Replace "107 " into "10⁷".
- 9) Delete Valencia, CA.
- 10) Add City and State name in Millipore.
- 11) Add City and State name in Eptomics.
- 12) Add City name in cwBiotech.
- 13) Remove USA.
- 14) Change t in t-test to *Italic* style in this manuscript.
- 15) Replace "Fig. 2B " into "Fig. 4B".
- 16) Replace "phosphor-ERK1/2" into "phospho-ERK1/2".
- 17) Replace "phosphor-ERK1/2" into "phospho-ERK1/2".
- 18) Replace "Fig. 4B" into "Fig. 4C".
- 19) Add the "PBX3 by Q-PCR".
- 20) Show bp and kDa for each gene in Fig 1A.
- 21) Modify the loading amount of proteins samples in Fig 1A.
- 22) Replace "Lentivirus Control" into "Lentivirus control" in Fig 2A.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

A handwritten signature in black ink that reads "Haibo Han". The signature is written in a cursive, slightly slanted style.

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