July 22th 2015

Name of Journal: World Journal of Gastroenterology

ESPS Manuscript NO: 19630

Title: Clinical significance of golgi phosphorylation protein 3 expression in colorectal cancer

Author: Yan-ta Guo, Cheng-zhi Qiu, Zhong-xin Huang, Wai-shi Yu, Xiao-feng Yang, Ming-zhen Wang

Dear Editor,

Thank you for your e-mail regarding above mentioned manuscript. We also appreciate valuable suggestions of the reviewer.

1. The manuscript has been improved according the Format for Manuscript Revision: Basic Study. Please find enclosed the edited manuscript in Word format.

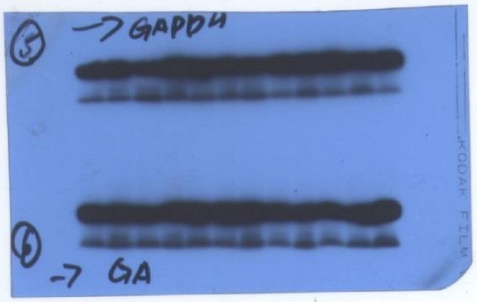
2. Revision has been made according to the suggestions of the reviewer.

1) The major concern with this manuscript is that the specificity of the

antibody to GOLPH3, and the authors make no attempt to validate this specificity. The antibody used has not been widely validated in the literature, and even the manufacturer's own data demonstrates that the antibody recognizes other (non-GOLPH3) bands on Western blot, and so this becomes a critical issue.

Response: The antibody to GOLPH3 used is a kind of polyclonal antibody, and its sensitivity is high. When we handled the results of Western blot, we cut the membrane into two pieces according to the location of the marker, one containing the GOLPH3, and another containing the GAPDH internal protein. The membrane containing GOLPH3 includes target band and nonspecific band, and we cut off the nonspecific band from the membrane. The following is our experimental results of Western blot and instructions about the instructions about Rabbit anti-GOLPH3 (ab98023) polyclonal antibody (Abcam).

Please see the following Figures: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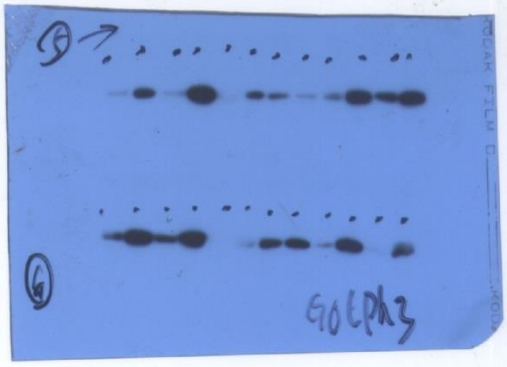
GAPDH
6
GA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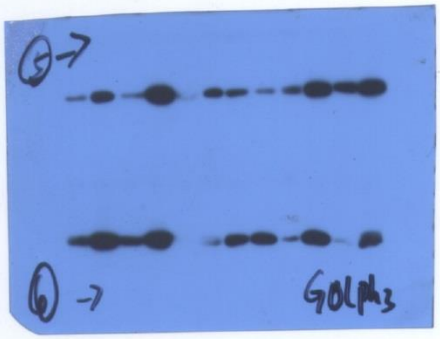
Golph3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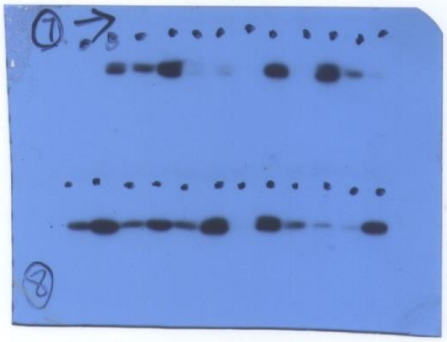
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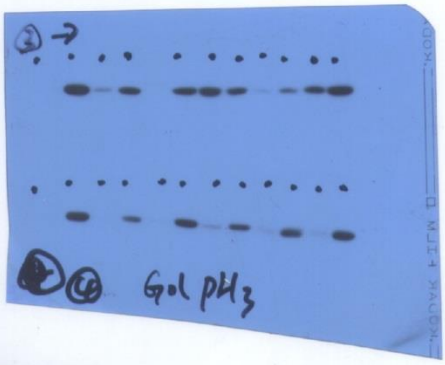
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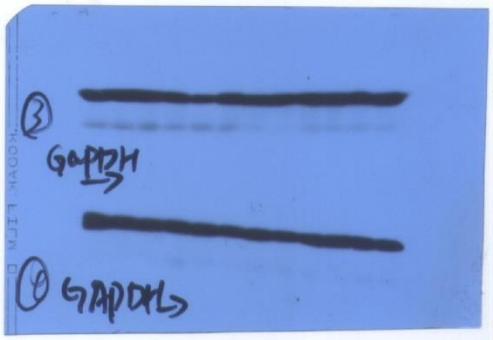
Golph3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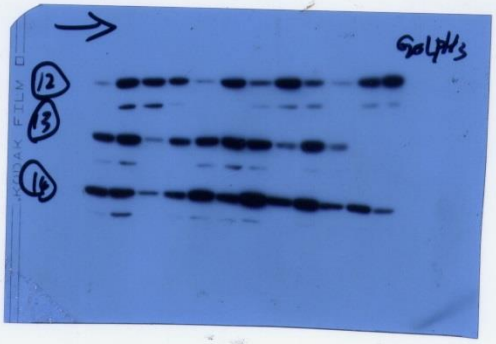
GAPDH
4
GAPDH


GAPDH3
12
13
14


GAPDH3
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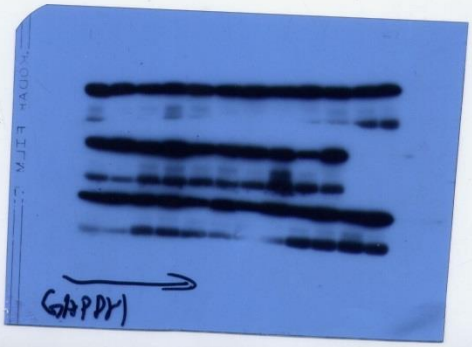
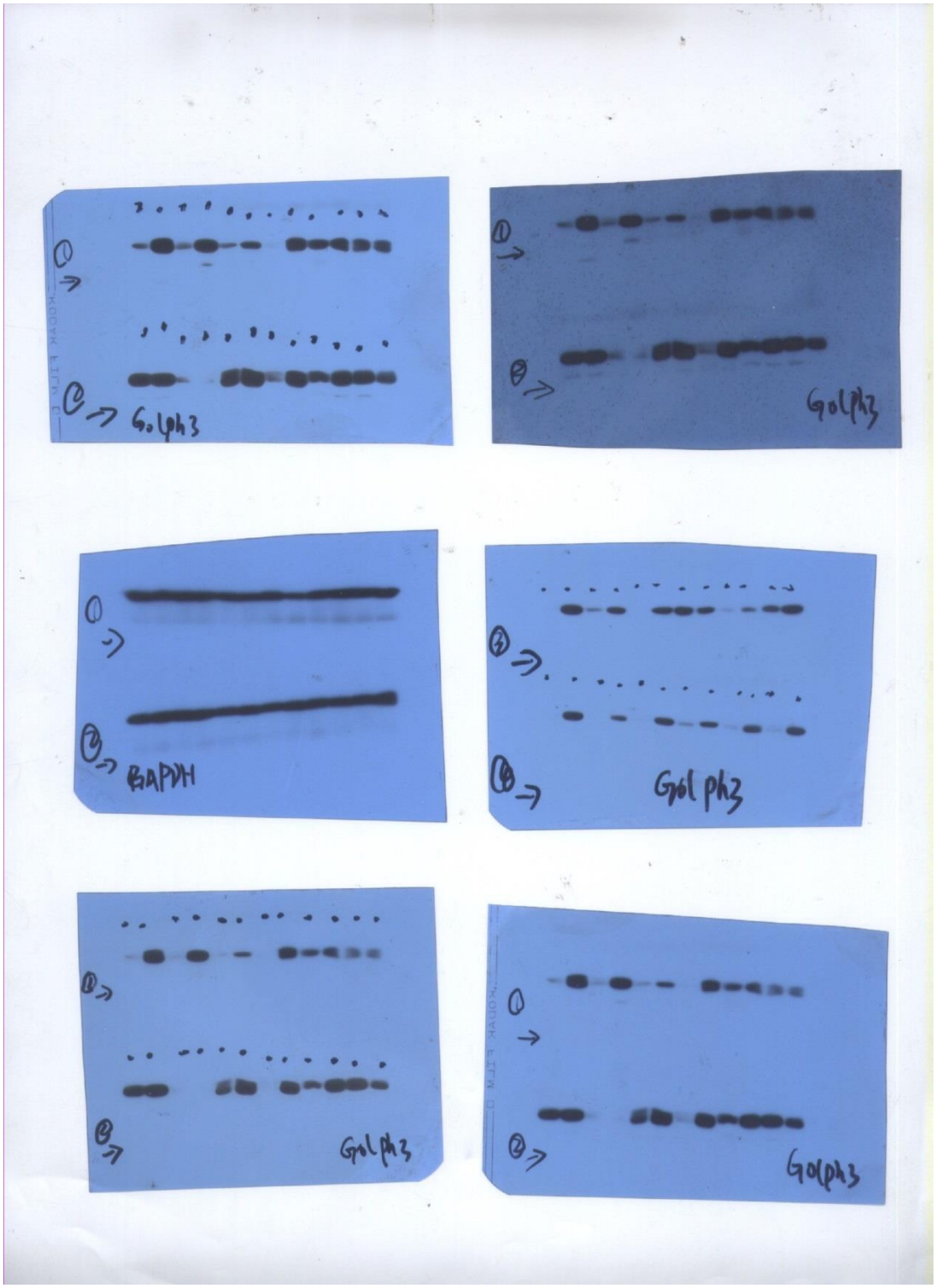

GAPDH1



2) Why was the expression of GOLGH3 and VEGF not determined utilizing semiautomated techniques?

Response: At that time, our image analyser was broken, so we could only

use manual counting method. We know the advantage of image analysis is that its judgement is more objective, and less artificial interference. Studies have shown that image analysis can achieve better judgement for cytoplasm and nuclei positive results.

3) The authors indicate pathology review of Immunohistochemistry studies was conducted by 2 blinded pathologists, any disagreements were resolved by consensus. Can the authors clarify this, and describe how consensus was defined?

Response: In the few instances of discrepant scoring, two pathologists will have a recount and take the average.

Your kind consideration of the revision for publication in World Journal of Gastroenterology would be greatly appreciated.

Sincerely,

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