Answers to Reviewers Comment

March 11, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 8082-review.doc).

Title: Cell-type specificity of β-actin expression and its clinicopathological correlation in gastric adenocarcinoma.

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Name of Journal: *World Journal of Gastroenterology*

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

1. Format has been updated and all the corrections are highlighted in red. Corrections are in line numbers 34, 38-41, 56-58, 62, 69-71, 89, 137, 158, 159, 160, 202, 203, 292, 317, 569, 570, and 577-579.
2. As per the editor's instruction the abstract has been reformatted and divided into Aims, Methods, Results and Conclusion.
3. As per the editor's instructions Background, Research frontiers, Innovations and Breakthroughs, Applications, Terminology of the article are included in the manuscript.
4. As per the editors instructions where P-values are expressed as mean ± SE.
5. A new bar graph with statistical analysis has been included in Fig. 2B with necessary corrections in the figure legend.
6. **Answers to the Reviewer's Comments:**

Reviewer 1:

We sincerely thank the reviewer for the kind and encouraging comments.

Reviewer 2:

Major Points:

The reviewer said: 1 "Paper used IHC and qPCR to test b-actin expression. This

altered b-actin could be caused by any of tumor inflammation reactions. There is not a cause-effect between increased gastric cancer and altered b-actin".

Reply: We thank the reviewer for the comment. Tumor microenvironment including inflammatory or infiltrating immune cells (lymphocytes, macrophages, neutrophils, eosinophils, and mast cells) goes through a constant evolution and promotes neoplastic transformation and invasion via paracrine factors[1, 2]. Inflammation is one of the hallmarks of cancer and several studies using human clinical samples have shown its effect on epithelial cell turnover[3, 4] and predisposes humans to carcinoma in the breast, prostate, liver, large bowel, gastric mucosa, urinary bladder, ovary, and skin[3-8]. On the other hand long-term use of nonsteroidal anti-inflammatory drugs (NSAIDs) and aspirin reduces the risk of colon cancer by 40–50%, and may be preventative for esophagus, lung, and stomach cancer[9, 10]. Therefore, inflammation has a definite cause-effect with cancer[11].

In gastric cancer inflammation plays a pivotal role being chronic gastritis an initial stage of cancer development which leads to atrophic gastritis →intestinal metaplasia→ Dysplasia→ gastric cancer. Therefore, there is a definite cause-effect relation between inflammatory reactions, altered β-actin levels and gastric cancer.

References:

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The Reviewer said: 2 "The results were over-stated and misinterpreted. In any points, paper needs more data and delicate experimental designs to study and draw the conclusion "relationship of increased b-actin and tumor biomarker".

Reply: While we thank the reviewer for this comment, however, we were unsure as to which data the esteemed reviewer perceived as being overstated and misinterpreted by us. We, the authors, understand the reviewer's concern regarding the low sample size as a limitation to confirm β-actin as a biomarker. However, along with our other observations, a significant correlation of β-actin and tumor grade in gastric cancer as shown by our study provides sufficient

rational to further explore β-actin as a biomarker in cancer but not to be used as an equal loading control.

In whole manuscript authors have mentioned β-actin as a biomarker only at three places *(1) in the end of the abstract (2) in the 1st para of discussion and (3)* in *the last para discussion.* At all three places authors have never made any direct statement; we have only expressed the possibility of β-actin to be considered as biomarker in cancer. However, on the reviewer's wish authors have changed the abstract lines from Collectively, our results showed a predominant β-actin over-expression in inflammatory cells, its cell type specific correlation with tumor grade and thereby suggesting β-actin as a potential tumor biomarker and chemotherapeutic target in gastric adenocarcinoma"

to

"In gastric cancer β-actin shows an overall higher expression predominantly contributed by inflammatory or tumor infiltrating immune cells of the tissue microenvironment and correlates with tumor grade."

The Reviewer said: 3 "The inflammatory cells are not categorized at all. Are they all tumor inflammatory cells in the Figure 2A"?

Reply: We thank the reviewer for the comment. The population of tumor inflammatory or tumor infiltrating immune cells is composed of mainly lymphocytes (NK, T ot B cells) which can be easily identified with HE staining because of their spherical shape with large nuclei and less cytoplasmic content[1]. These cells have immense prognostic value in gastric as well as in other cancers[1-4].

In a gastric tissue micro-environment, our study has identified inflammatory or infiltrating immune cells and epithelial cells were majorly stained for β-actin and inflammatory cells predominantly contributed in overall high level of β-actin. Similar staining intensity of the all inflammatory cell of tissue microenvironment did not prompt us for their further categorization into the type of lymphocytes.

However, authors agree with the concern of reviewer and believe that further studies classifying inflammatory cells of the tumor microenvironment can provide interesting information about β-actin expression in specific type of inflammatory cells and cancer clinical behavior.

The reviewer has also asked that 'Are they all tumor inflammatory cells in the figure 2A? Authors want to ensure that all the spherical cells visible in in Fig. 2A (shown by blue arrow) are lymphocytes or inflammatory cells present in the tumor microenvironment.

Reference

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American Society of Clinical Oncology 2013; ***31****(7): 860-867 [PMID: 23341518 DOI: 10.1200/JCO.2011.41.0902]*

Minor points:

The Reviewer said: 1 "The gels in the Figure 1B and Figure 3C have poor quality".

Reply: We thank the reviewer for the query. However, we wish to point out that there is no gel picture in Figures 1B and 3C. Figure 1B shows differential β-actin level in normal and tumor tissue samples by western blotting; upper panel image of the figure 1B consists scanned image of X-ray film (β-actin and PVDF membrane). Figure 3C is a bar graph and not a gel picture.

Reviewer has said that the above mentioned figures have poor quality without indicating the problem. In the submitted manuscript (8082) al the figures is of 300 dpi, a standard resolution for most of the journals. Therefore, authors don't agree with the reviewers comments.

The Reviewer said: 2 "Figure 3B has no statistical analysis".

Reply: We thank the reviewer for the comment. Statistical analysis has been done for Fig 2B using normalized (β-actin western blot band intensity/ intensity of total protein lysate lane on PVDF membrane) values of three independent experiments. Unpaired t-test was applied.

Reviewer 3:

The Reviewer said: 1 "Inflammatory cells should be assessed by IHC. The authors should clarify which type of inflammatory cells expressed β-actin".

Reply: We thank the reviewer for the comment. Authors reply to the question is same as given to reviewer 2 for major point 3.

The Reviewer said: 2 "According to the results, they concluded that β-actin

might be chemotherapeutic target. Why? This must be overstatement. There are some exaggerated parts in this paper".

Reply: We thank the reviewer for the query and comment. Based on our observation and earlier studies (Ref. 17, 18 and 19 of the manuscript) we have only expressed the possibility of β-actin as a direct or indirect target for cancer chemotherapy. It's a well-known fact that reorganization of actin cytoskeleton accompanies oncogenic cellular transformation which is characterized by unrestricted proliferation, inappropriate cell survival and acquisition of the motile and invasive phenotype. Reversal of malignant phenotype by restoring actin filament stabilizing proteins implies direct role of actin cytoskeleton in oncogenic signaling. Based on these studies actin cytoskeleton, therefore, represents a point for chemotherapeutic intervention.[1-3]

The idea of targeting actin in cancer chemotherapy is not new, however, despite a number of actin targeting compounds the field is moving very slow because inability of existing anti-actin agents to discriminate between the actin cytoskeleton of tumor cells and the actin filaments of the muscle sarcomere. Therefore, targeting actin regulatory proteins which organize cellular function specific actin cytoskeleton and altered in cancer may specifically hamper actin cytoskeleton dependent function in tumor cells. Tropomyosin[4, 5], F-actin capping protein CapG[6] and Actinin-4[7] are examples of few actin binding proteins which have shown altered expression in cancer and studied as chemotherapeutic target disrupting actin cytoskeleton.

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7. References and typesetting were corrected

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

Sincerely yours,

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