Author's response to reviews

Title: Src activity is modulated by oxaliplatin and correlates with outcomes after hepatectomy for metastatic colorectal cancer

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Author's response to reviews: see over
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Dr. Richie Soong
Senior Editor
BMC Cancer

Dear Dr. Soong,

Please find enclosed our revised manuscript entitled “Src activity is modulated by oxaliplatin and correlates with outcomes after hepatectomy for metastatic colorectal cancer,” which I resubmit on behalf of my co-authors. We appreciate the helpful comments and suggestions for improvement of the manuscript by the reviewers. In response, we have modified the manuscript as detailed below.

Comments from Editor:
The article presents interesting and novel human tissue-based evidence of a role for Src activation in oxaplatin resistance As highlighted by Jun Zhang, the rationalization of Figures with the text is a must. The disconnection was quite concerning.
Greater disclosure of cohort demographics and associations, clarification of methods (e.g. not just "Qiagen kit"), and data (e.g. Oncocarta) is also required The authors should also provide support for the validity of the immunohistochemical scoring system It is hard to gauge how the first and second sample series concur or differ in their scores and associations.

We apologize for the confusion created by the numbering of figures with the corresponding citation in the manuscript text. The problem stemmed from the fact that our initial manuscript had five figures – labeled “Figure 1,” “Figure 2a,” “Figure 2b,” “Figure 3,” and “Figure 4.” However, when they were uploaded onto the journal website, they were instead labeled “Figure 1, 2, 3, 4, 5” such that a mismatch was created between the names in the manuscript and the corresponding file labels. To unify these figures with the text, we have changed “Figure 2a” to “Figure 2,” and “Figure 2b” to “Figure 3.” Likewise, “Figure 3” has now become “Figure 4,” and “Figure 4” has become “Figure 5.” This has been corrected in the manuscript as well.

We appreciate the recommendation to report the associations between cohort demographics. We added a Supplementary Table 2 in which distributions of gender, ethnicity, site of primary tumor, histological subtype of tumor, and tumor grade were calculated. These analyses were performed and showed no differences not only between the two cohorts of patients (N=120 and N=25) but also between the groups given different preoperative chemotherapies (oxaliplatin, irinotecan, or none) in the cohort of 120 patients. No statistically significant differences were observed for any metric measured.

We likewise appreciate the editor’s suggestion of clarification of the methodology. To that end, we have provided in the methodology the name of the specific platform used for sequencing of point mutations, and we added a supplementary table listing all point mutations tested in this series. Likewise, we provided the name and company of the materials used for DNA extraction from tissue specimens in the methodology section.

We acknowledge the lack of clarity in our description regarding the validation of the immunohistochemical scoring system. Both sets of tissue microarrays were scored for protein expression using the same quantitative scoring system
from Aperio Technologies, Inc. (Vista, CA; U.S.A.). We have clarified this in the methodology section and explicitly stated that the same quantitative scoring system was used for both sets of tissue microarrays.

Comments from Referee #1:
It has been reported that the nonreceptor tyrosine kinase Src could be activated after oxaliplatin exposure and in acquired oxaliplatin both in colon and gastric cancer. In this manuscript, the authors retrospectively evaluated the activation of Src and FAK in hepatic metastases of colorectal cancer and correlated these findings with the clinical outcomes of patients treated with oxaliplatin. Although the concept of Src activation after oxaliplatin exposure is not new, the Src activation correlated with poor patients' clinical outcomes is interesting. Beside these, the reviewer also has the following concerns:
1. The authors claimed that the Figure 2A were the levels of pSrc, Src, pFAK, and FAK in liver metastases treated with various chemotherapeutic regimens, but the data about Figure 2A could not be seen in the manuscript. The authors should provide them.
2. The authors showed the data named Figure 5, but there were no analysis about them both in the manuscript and figure legends.
3. In the results section, the authors described the correlation of Src and FAK with gene mutations and PTEN expression, the data could not be found in the manuscript about this except some P value.

Comments 1-2: We thank the reviewer for the comments. As mentioned above, in the initial submission, what the electronic submission had recognized as “Figure 5” was described in our manuscript as “Figure 2A.” Therefore, there was no mention of “Figure 5” in the text of our manuscript because we had been referring here to “Figure 2A,” just as the data we had been describing as “Figure 2A” had been provided by “figure 5.” We acknowledge the error made in uploading the figures initially onto the journal's website, and again apologize to the reviewers for the inconvenience and confusion this may have caused. All figures have been renamed without any “A” or “B” subset and now match between the text of the manuscript and the corresponding figures.

Comment 3: We have added a Supplementary Table 3 to the end of the document which quantifies the pSrc/Src and pFAK/FAK ratios and lists the corresponding p-values. We also included these ratios in the body of the manuscript as well for those oncogenes for which a significant difference was observed in the ratios between the wild-type and mutated cohorts.

Comments from Reviewer #2:
There is no specific deficiency in need of specific revision. The paper is mostly descriptive. The conclusions are soft at best. In the discussion, there is the usual but acceptable suggestion that the soft correlations found are potentially clinically meaningful and biologically plausible. However, in doing so, the tone well balanced. There are caveats on what prior treatment means and on the ability to draw any conclusions around oxaliplatin vs irinotecan vs no prior chemo --and on what the "validation" cohort vs simply some more pts really means.

We appreciate the feedback from this reviewer and agree with his concern that the second cohort of patients was not a “validation” cohort. To clarify this point further, we have substituted the word “additional” in place of the word “validation” throughout the body of our text.
We hope that the revisions above should address the issues raised by the editorial office and reviewers and have improved the manuscript. We hope that you will now find the revised manuscript suitable for publication in BMC Cancer.

Sincerely,

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The University of Texas, M.D. Anderson Cancer Center