Author’s response to reviews

Title: Interaction of a traditional Chinese Medicine (PHY906) and CPT-11 on the inflammatory process in the tumor microenvironment

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Author's response to reviews: see over
March 7, 2011

Dear Editor of BMC Medical Genomics:
Thank you for your favorable review of our manuscript MS: 1578451354498788
Interaction of a traditional Chinese Medicine (PHY906) and CPT-11 on the inflammatory process in the tumor microenvironment by Ena Wang, Scott Bussom, Jinguo Chen, Courtney Quinn, Davide Bedognetti, Wing Lam, Fulan Guan, Zaoli Jiang, Yichao Mark, Jeffrey White, Francesco M Marincola and Yung-Chi Cheng. We thank the reviewers for their positive and constructive remarks. We believe we addressed most of the concerns/requests and we hope you will find the manuscript acceptable for publication in the present format:

In detail:

**Reviewer #1**
This is an article important in its field. It gives detailed scientific evidences for a recently published article: Ewen Callaway, "How an 1,800-year-old herbal mix heals the gut", 19 August 2010, Nature News, doi:10.1038/news.2010.417. The authors have done comprehensive experiments and statistical analysis to support their results, including pathway analysis, clustering on differentially expressed genes, etc. The article is good to publish. As the experimental results are the primary contributions of this paper, please make sure the essential experimental results are repeatable. **We thank you the reviewer for these comments.**

**Minor Essential Revisions:**
1. It is better to cite the article "How an 1,800-year-old herbal mix heals the gut" in the introduction. **The reference was added as requested**
2. On page 5, last line of the first paragraph. "Nevada Cancer Institute" is repeated. Please delete one. **Deletion was done**

**Discretionary Revisions:**
There are some ways to improve the paper if authors would like to. For example, to create a gene correlation network for each microarray data, and study their difference/similarity using network mining algorithms/ tools. **We feel that the manuscript is sufficiently dense in information that further analysis would not be easy to incorporate; we want to emphasize that the manuscript is focusing on specific immunologic/pro or anti-apoptotic effects of the treatment while another analysis is being performed with a broader scope to describe the general effects of the combination and for this second manuscript we will use the analysis suggested by the reviewer.**
Reviewer #2

The authors have investigated how a traditional Chinese medicine (PHY906) exerts antitumor effects during chemotherapy in an established clinical model through microarray expression profiling. This study is novel and may draw wide readership because PHY906 demonstrates anti-inflammatory effects when administered alone in vivo. The author’s principle finding is that co-administration of PHY906 with a chemotherapeutic agent exerts a pro-inflammatory, pro-apoptotic effect in a cancer cell-specific manner. The microarray study is well designed and the abstract clearly conveys the author’s findings and their wider implications, but the presentation of the actual results and the explanation/justification of the analytical strategies employed are often confusing to the point of frustration. Even if one is to assume the analytical strategy is sound, the results as presented, in the reviewer’s opinion, could be easily interpreted by a limited number of readers. Furthermore, interpretation of the results is problematic as the authors have performed no experimental validation of the microarray data. The authors have, however, experimentally validated an immunologic effect suggested by pathway analysis, immune cell infiltration in tumors. We thank the reviewer for the positive overall assessment of the work; the rebuttal to criticism is presented in the following paragraphs where the reviewer goes into detail about specific changes.

Major Compulsory Revisions:

1. The order of and manner in which gene expression data and pathway analyses are presented requires the reader to constantly refer back and forth between multiple results sections and figures. As the study and data are complex (effects of three factors on three tissues with cross-comparisons), the authors should make a thorough revision of the results section. Starting at section two, a more effective and coherent order of presentation would be “Comparison between the tumor, spleen, and liver”, “Tumor”, “Effect of CPT-11”, “Effect of PHY906”, “Effect of PHY906 when combined with CPT-11”, “PHY906-specific effects during CPT-11 therapy”, and “validation of immune cell infiltration in tumor by immunohistochemistry”. We respectfully disagree with the reviewer about the order of presentation, the most salient changes are due to the CPT and, therefore, we would like to present the minor effects of the herbal product first and then focus on the modulatory property of the herbal product on the chemo. I hope this will be acceptable. About the interactive presentation between text and figures, we think that could be best addressed by the layout of figures which hopefully will be presented intermixed with text at the most appropriate time; we believe this would be the best solution. We agree that the presentation is complex but we really are at a loss about trying to increase its understanding without the utilization of figures as a tool. I hope this can be addressed at the editing stage.

2. The data analysis packages BRBArray Tools and Ingenuity Pathway Analysis were both designed, in part, to allow experimental scientists to perform sophisticated computational analyses typically done by biostatisticians and bioinformaticians. Given that (a) the authors used these tools, (2) none are affiliated with a statistics or computational science department, and (3) there is no acknowledgement of their having consulted a statistician or biomedical informatics core facility at their respective institutions, the authors should do so now and include such an acknowledgment in the manuscript or provide justification as to why they conclude this is not necessary. We have been involved in array analysis since its origin more than a decade ago and two of the authors of the manuscript have actively participated in the development of BRBArray analysis. Our group has worked for a decade with Richard Simon’s group and we have limited their involvement for straightforward analyses such as the ones presented here; in any case, we asked Dr. Yingdong Zhao, from the NCI biometric branch to review the data and validate our results; his name was added to the authors’ list.
3. The authors should experimentally validate some of the gene expression data by an independent method, such as qRT-PCR. This should include expression of Mcp-1 and Irf1. Simply plotting microarray data for a gene (as in Figure 5E) is insufficient. **Validation of several genes by qPCR was added to the manuscript as requested (figure 6); however, we left the description of Mcp-1 in figure 5E just for display purposes to guide the interpretation of the histological data.**

**Minor Essential Revisions:**

1. The first page of the manuscript lists supplementary data as being available at Cancer Research Online. **This inaccuracy was removed**
2. On page 7 Results paragraph 2, the authors describe “7,856 transcripts differentially expressed by tumors compared to the two normal tissues”. To be clear, does this mean both or either normal tissues? **This analysis refers to comparison between the tumor and the two normal tissues combined and it has been clarified in the text.** If expression patterns are distinct in tumors versus the normal tissues, between the normal tissues themselves, and drug responses are also different among tissues, why combine liver and spleen (Figure 2, legend)? **This presentation aimed at emphasizing differences between tumors and representative normal tissues pooling information from two normal tissues, by providing specific information for each tissue we believe we would further complicate the data beyond the purpose of the point we are trying to make emphasizing commonalities among normal tissues compared to tumor. Obviously, the point the reviewer is making is important and we are happy to share in the future specific information about differences between liver and spleen but we believe that this information is excessive for the purposes of this manuscript.**
3. The syntax of citing of p-values for Fisher’s exact test is inconsistent. See page 9 Results paragraph 4. **The imprecision was corrected; all Fischer’s test p values are presented as two-tailed.**
4. The powerpoint file containing Figure S1A does not have a file extension, at least on the BMC website. **This was corrected, thanks for pointing this out.**
5. The axis values on Figure 2 are illegible. This makes data interpretation difficult. The authors should export the file at a higher resolution; 300 dpi, compressed tiff may be appropriate. They may also consider checking whether IPA allows one to increase font sizes. **The Axis labels were photoshoped over the original to enhance resolution (files had been extracted at 600 dpi but the fonts could not be changed).**
6. I somehow doubt this is the first study in which the effect of chemotherapy was assessed at the global transcript level. See page 14, Discussion paragraph 1. **We have not found any previous study in which the effect of chemotherapy has been studied in vivo with high throughput technology. It is sort of sad but we have been advocating this kind of approach for years and wrote several editorials about this problem. On the other hand we agree that the statement is superfluous and somewhat gratuitous and it was removed.**
7. Why are data being introduced in the Discussion section? See page 16 Discussion paragraph 4, Data S2. **The data are part of another study and are provided as supplemental material (S2); are not really directly related to the study and are not presented in the results section but are referenced for discussion purposes and provided as a supplement. We believe that this may be a reasonable approach.**
8. There are several grammatical errors in the manuscript. **The manuscript was edited by a native English language speaker and several grammatical errors were detected and corrected**

**Discretionary Revisions**

1. The second sentence of Discussion paragraph 6 on page 17, “the study ... documentable effects” is rather general. **The entire sentence is redundant with the preceding and following ones and was removed completely**
2. The headings of the Results section are not very descriptive. For example, “Tumor” or “Effect of CPT-11”. **The headings were expanded and clarified**
3. CPT-11 is referred to as Irinotocan in legend of Figure 4D and nowhere else in the manuscript. We thank the reviewer for the careful analysis of the manuscript. This and other inconsistencies that could be identified have been corrected.

Reviewer # 3

Reviewer’s report:
The manuscript by Wang et al performed microarray and IPA analysis on tumor tissue treated with CPT-11 and/or traditional Chinese Medicine PHY906. The result which is very interesting, however lacks validation by independent methods such as qRT-PCR. Again thank you the reviewer for the positive comments.

Major Compulsory Revisions
1. The authors should perform qRT-PCR on few targets such as genes that are over-represented in NF-kB pathway in which IPA found that. qPCR validation of several genes was added as Figure 6.
2. The authors have examined the infiltration of macrophage in the tumor tissue. How about CD8 +ve cytotoxic T cell? Should the authors examine the effect of TCM on CD8 cells also? The changes studied were early in the treatment and, as we previously observed in other models, most of the changes involved innate immune responses rather than adaptive ones which follow a few days later, none of the genes that were identified suggested localization of T cells at this time point although the reviewer is correct that they should be expected to be there though, we believe, at a later time point. This is why no analysis of CD8 cells was performed.

Editor’s Requests:
- The manuscript was copy-edited by a colleague native English speaker
- The abstract was formatted according to the Journal Style
- The data was deposited in Gene Expression Omnibus

Finally, Dr David F Stroncek who helped in the design of the array studies and their interpretation had been omitted in the previous submission and was added as a co-author

I hope you will find now suitable for publication in BMC Medical Genomics

Thank you again for your consideration

Sincerely,