Author's response to reviews

Title: Molecular Pathways Undergoing Dramatic Transcriptomic Changes During Tumor Development in the Human Colon

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Version: 2 Date: 14 June 2012

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The Biomed Central Editorial Team

Object: MS: 3241921196862096 - Molecular Pathways Undergoing Dramatic Transcriptomic Changes During Tumor Development in the Human Colon. Rosalia Maglietta et al.

Thank you for being interested in our manuscript. Encouraged by the positive assessment of the reviewers and according to your suggestion of resubmitting the manuscript to BMC Cancer journal, we have prepared a new version of the manuscript in which we have addressed all the remarks and doubts raised by the referees.

Reviewer #1 (Davide Cora’)

Major Compulsory Revisions

1) The authors discussed a set of transcriptomic profilings (GeneChip Human Exon 1.0 ST array), however there is no validation at all of the data in this manuscript. Could the authors add some validation of the data they used or, alternatively, indicate where interested readers could find such information?

We have addressed this comment on pages 6, 21, and 22.

- Page 6: a new sentence in the Background section now appears as:
  “Because the preinvasive stages have been far less extensively explored than the cancerous phases of this process, there were no independent sets of transcriptomic data on precancerous lesions that we could use to validate our findings. To overcome this limitation, we used two strategies. Firstly, we re-analyzed all of our original data sets with GSEA and compared the results with those obtained by using RS; farther, our findings obtained comparing CRCs and normal colorectal mucosa were subjected to additional validation based on the application of RS method to two publicly available data sets on these types of tissues.”

- Page 21-22: the validation analysis has been described in a new section of the manuscript entitled Validation of Experimental Results.

2) The authors used the RS method to investigate their gene-expression profiling. Is it possible to add a “computational” validation for the results presented here? For example, is it possible to use the GSEA method and retain for the discussion only pathways confirmed by at least two independent methods (RS + GSEA)?

We added the required “computational” validation on pages 10, 21, and 44.

- Page 10: we added this sentence in the Statistical methods:
  “The validation procedure involved the use of standard GSEA [16], and p-values for the enrichment scores were computed on the basis of 1000 label permutations.”

- Page 21: as suggested by the reviewer, we have applied GSEA for analyzing the preinvasive and cancerous phases of the colorectal tumorigenesis process in
terms of pathways and compared the results with the ones obtained with our RS enrichment approach. The discussion of the validation was discussed in the new section Validation of Experimental Results.

- Page 44: table 4 shows the numbers of pathways displaying significant tumor-associated dysregulation in RS and GSEA analysis.
Reviewer #2 (Iris Simon)

Minor points

1) Do have patients follow-up? With other words, are there indications that some tissue samples came from aggressive or less aggressive cancer that formed metastasis or new tumors in short time and are in these samples cancer-driving pathways stronger?

The patient follow-up is not available.

2) Results and Discussion section is difficult to read as it jumps between different unrelated pathways. Figure 4 is very helpful and maybe can be used as a guide for this section.

According to the suggestion of the reviewer, we have modified the Results and Discussion section focusing on the main pathways found deregulated in the different phases of the tumorigenesis process. Figure 4 was modified.

Suggestions for improvement

1) Focus the result section on those pathways that are well understood (as it has been done for example for the cell-cycle genes/ Figure 2)

No further comment because this remark has been addressed previously.

2) Validate results in publically available datasets (this is possible at least for results in cancer patients)

We have addressed this comment on pages 21, 22, 45.

- Pages 21-22: the deregulated pathways obtained comparing CRCs with normal colorectal mucosa were additionally validated by using RS method applied to two publicly available data sets. The discussion of the results was made in the new section Validation of Experimental Results.
- Page 45: table 5 shows the numbers of pathways displaying significant tumor-associated dysregulation in RS analysis of the N vs CRC data set and in two independent validation data sets.

3) Sharpen the conclusion section. At the moment it is more a discussion. What are the major new results? How does this analysis helps a patient or a physician to better deal with the disease?

Page 22: in the conclusions we added the sentence:
“This exhaustive description of the sequence of critical molecular events characterizing the progression of colorectal tumors is based on a statistically robust analysis of transcriptomic data carried out at the level of functional molecular processes, rather than individual genes or proteins. This analysis was able to reveal specific pathways
characterizing each transition of the tumorigenesis process. More importantly, these findings were independently confirmed by a different statistical method and validated by two publicly available data sets relative to the cancerous phase."