Author's response to reviews

Title: Analyzing the regulation of metabolic pathways in human breast cancer

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Author's response to reviews: see over
Heidelberg, July 5th, 2010

Dear Editor,

This is the second revised version of our manuscript “Analyzing the regulation of metabolic pathways in human breast cancer” (manuscript MS: 9233126683311198). We would like to thank the reviewers for their helpful comments. With them we could improve the manuscript, specifically by reducing the spelling and grammar errors. We also added the ROC-curve for simulated data.

Please find below a point by point response in which the comments of the reviewers are listed together with our respective remarks and references to the corrections in the paper. For clarity, we enumerated all points.

We think that we well addressed all comments of both reviewers. If you have got any queries do not hesitate contacting us,

Best regards, Rainer König

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Point-by-point response

Reviewer: 1

Major compulsory revisions:

1. My major comment remaining is that they need to compute receiver operating characteristic curves for the simulation study (i.e. vary the p-value cutoff and find the new values of sensitivity and specificity).

   As suggested by the reviewer, we computed the ROC-curve for Fisher’s exact test, GSEA and our approach using simulated data and varying the p-value cutoff between 0 and 1. The curve is shown in the Supplement S2 and we refer to it in the main manuscript in the end of Results and Discussion

   (“We compared the performance of our method to Fisher’s exact tests and GSEA with simulated data yielding considerably higher sensitivity for our method (see Supplement S2 and receiver operator characteristics: Figure S2).”)

Reviewer: 2
1. The paper was improved according to my previous comments, and it is ok now. I just came across a few typo's and mistakes that the authors may wish to change.

*We thank the reviewer to point to many spelling and grammar errors. We corrected all typo's and mistakes.*

2. Background, first paragraph:
"70-80% of the patients would have survived without [chemotherapy]" --> for how long?

*We specified the survival time, page 3, 2nd paragraph: “However, 70-80% of the patients would have survived without it (five years of follow-up).”*

*Additionally, we added two more references for this statement, i.e.:


3. *The following corrections were made:*

"remains to be poorly understood" --> "remains poorly understood"

"due to incompleteness or erroneous in the available data" --> erroneous use of the word erroneous.

4. The first paragraph of the background section should be split up into smaller paragraphs.

*We split the first paragraph of the background section into smaller paragraphs to improve readability.*

5. *The following spelling errors were corrected:*

Results and Discussion:

"dense packed reactions" --> "densely packed reactions" (unless I am unaware of an alternate meaning of "dense packed reactions") "were preserved as optimal as possible" --> "were preserved as optimally as possible"

6. Pathways for energy supply were significantly up-regulated: "Glycolysis is increased in cancers to generate ATP" --> as far as I'm aware of, all we know is that glycolysis is increased in cancers and that this generates ATP. Any other connection between these facts would be speculative. Also, in this paragraph, other assumptions about the Warburg effect are made.
The reviewer is correct that the up-regulation of glycolysis in tumors is still not completely understood and some caution is necessary. Therefore, we rephrased the beginning of the section “Pathways for energy supply were significantly up-regulated” (page 7, lower part):

“We detected significant differential expression patterns in glycolysis ($P=3.85E-03$), pyruvate ($P=1.20E-02$) and fructose/mannose ($P=1.55E-02$) metabolism. They were mostly up-regulated to generate sufficient energy and metabolites for fast-growing cancer cells. Genes of the glycolysis pathway have been found to be over-expressed in a set of 24 cancers, while other pathways showed less consistent up-regulation so far [22]. Glycolysis is increased in cancers and this generates ATP by oxidative phosphorylation [23, 24]. Furthermore, elevated glycolytic flux may support the production of important metabolites that contribute to essential cellular processes like fatty acid synthesis, nucleotide synthesis and to the better protection of oxidative stress [25, 26].”

7. The following spelling errors were corrected:

"extend" --> "extent"

"analogue" --> "analog" (analogue is British English, the rest of the manuscript is in American English)

8. This section could be split up into smaller paragraphs to improve readability.

We have split the section into smaller paragraphs.

Biosynthesis of steroids and bile acids:

9. The following spelling error was corrected:

"elevated concentration of DCA was detected" --> "elevated concentrations of DCA were detected"

10. This section could be split up into smaller paragraphs to improve readability.

We have split the section into smaller paragraphs.

Analyzing a second dataset

11. The following spelling errors were corrected:

"12 pathways with significant differentially regulated patterns" --> "12 pathways with significantly differentially regulated patterns"

Conclusion:

"in respect to" --> "in order to" (?)
Preparing the microarray data:

"except of" --> "except for"

12. In the text, "ambiguous samples" are defined as those with a time recurrence <3 years without metastasis. No mention is made of samples with a time recurrence >3 years but <5 years. How were these treated, if they existed?

We didn’t use these samples. We clarified this in the manuscript page 12, last sentence in section “Preparing the microarray data”:

Samples with ambiguous information were discarded (45 samples) and identified either by a time recurrence of less than three years without the event of metastasis or by a time recurrence above three years but below five years.

13. The following spelling error was corrected:

References:

21: "Glykolysis --> glycolysis"