Author's response to reviews

Title: Transcriptional Changes Associated with Resistance to Inhibitors of Epidermal Growth Factor Receptor Revealed Using Metaanalysis

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Author's response to reviews: see over
Dear Drs. Barbosa-Morais and Relox,

Thank you for taking care of our manuscript MS: 1263771791523977, “Transcriptional Changes Associated with Resistance to Inhibitors of Epidermal Growth Factor Receptor Revealed Using Metaanalysis.” The reviewers had important comments regarding our submission, which we are addressing in this letter and making changes in the manuscript.

Specifically:
1. The authors performed their analysis on each type of inhibitors. However, because for different inhibitor types, the cell lines used are also different, is it possible that the specific ontology terms observed for each type of inhibitors is actually related to the cell line?

   The cell lines we analyzed included non-small cell lung cancer, head and neck cancer, epidermoid carcinoma cell lines and skin cancer line (Table 1). We have considered the possibility that the results observed for each type of inhibitors are actually related to the cell lines and addressed it in the discussion section of the manuscript: “we note that both GSE34228 and GSE38310 compared Non-Small Cell Lung Cancer lines, so the characteristic differences between Gefitinib- vs. Erlotinib-selected resistance cannot be ascribed to different cell types.” Note also that the resistant cell lines were derived multiple times (10 different resistant cell lines, Table 1); our metaanalysis provides the overall view of the resistance phenotype, describing the commonalities, we were not comparing individual resistant lines. Additionally, we performed analyses on individual studies separately, essentially re-analyzing the original data as did the original authors. Therefore we concluded that the differences are related to different inhibitors, not different cell lines.

2. The method section is not clear enough. Especially, the authors should present how they normalized the non-Affymetrix datasets, and describe a little more about how the microarrays from multiple studies are treated in the RankProd software.

   In the Methods section, we have added the following phrase “For non-Affymetrix studies, where we could not run RMAExpress quality control, we downloaded already normalized, _RAW.tar files and used these without further modifications, as submitted by the original authors.”

   We also added the following description of the RankProd software: “RankProd package analyses gene expression microarray data specifically to identify differentially expressed genes. RankProd uses non-parametric rank product method to detect genes that are consistently found among the most strongly upregulated ones and the most strongly downregulated ones in a number of replicate experiments, comparing two different condition”
3. The legends of the tables should be clearer.
   We have expanded legends to all tables as well as to Fig. 2, explaining our approach and what the categories represent.

4. (Reviewer 2)...Therefore, the meaning of EGFR-TKI in EGFR-mutant lung cancer is different from that of EGFR-TKI in EGFR over-expressed cancers including head & neck cancer and epidermoid carcinoma. It is also different from the meaning of EGFR blocking antibody both in EGFR-mutant lung cancer and EGFR over-expressed cancers. So, the rationale of putting these together into one analysis does seem a bit far-fetched, considering the different context. It would be better to focus on the resistance to EGFR-TKI in lung cancer with EGFR mutation or the resistance to EGFR blocking antibody in head & neck cancer with EGFR over-expression.

We completely agree with the reviewer that the EGFR mutant vs. EGFR overexpressing tumors represent different categories. However, in this work we were focused on the transcriptional mechanisms of development of resistance to EGFR inhibitors, which should have commonalities in the two categories. It would be of great interest to compare the EGFR-mutant vs. EGFR-overexpressing tumors directly (for which at the moment there is insufficient data to afford metaanalysis), as well as separately comparing the effects of EGFR inhibitors (which we addressed in our previous paper, PMID:25184905), but here we are specifically concerned with the effects of resistance. While perhaps far-fetched, the metaanalysis, in our opinion, provides important new knowledge and understanding of the differences between the cells resistant and sensitive to EGFR inhibitors.

In this letter and in changes in the manuscript itself all reviewers’ comments are addressed. The manuscript was proofread again, making minor changes. The changes in the manuscript are noted by underlining. With these revisions, my coauthors and I hope the manuscript is acceptable for publication in the BMC Cancer.

Cordially yours,

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